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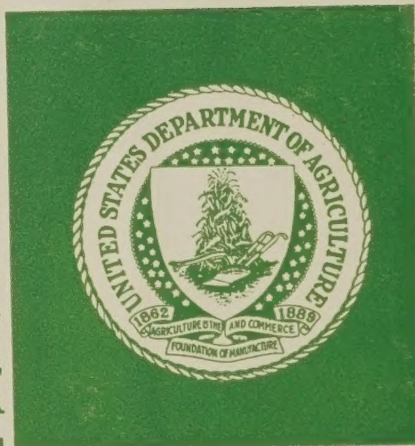
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OCT 5 1982

SUBJECT: Final Report for Specific Cooperative Agreement 58-519B-0-899,
University of Nebraska

TO: Technical Information Systems
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Division of Acquisition
Beltsville, MD 20705

Enclosed are two copies of the final report resulting from a Specific Cooperative Agreement Number 58-519B-0-899 between the University of Nebraska and ARS.

The Principal Investigator is Rodney D. Allrich and the ARS's ADODR is R. K. Christenson, Clay Center, Nebraska. The funding level was \$10,500 for an 18-month period.

The subject of the agreement is "Investigate the Biological Basis of Sexual Maturation in the Pig."

CONNIE J. MOORE
Head, Research Agreements

Enclosure

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U.S. Meat Animal
Research Center

P.O. Box 166
Clay Center
Nebraska
68933

July 19, 1982

SUBJECT: Overview on Final Report of Specific
Cooperative Agreement No. 58-519B-0-899

TO: P. J. Fitzgerald
Regional Administrator, NCR
Peoria, IL

THROUGH: C. W. Alexander *Robert E. Oltjen*
Mid-Great Plains Area Director
Columbia, MO

One of the pleasures of my position during the past few months has been to review the final progress reports of our predoctoral graduate students who have completed their Ph.D. research here at the MARC and their course work at a land grant university.

This program, initiated at the MARC about three years ago, has brought immense benefits to the taxpayer and is perhaps one of the best uses of funds that I know of!

ROBERT R. OLTJEN
Director

cc:
R. K. Christenson
J. J. Ford
C. J. Moore ✓

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Agricultural Research
North Central Region
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U.S. Meat Animal
Research Center

P.O. Box 166
Clay Center
Nebraska
68933

July 14, 1982

SUBJECT: Final Report of Specific Cooperative
Agreement No. 58-519B-0-899

TO: Dr. Paul Fitzgerald
Regional Administrator
USDA, SE, ARS, NCR
Peoria, IL

Connie J. Moore
Section Head, Contract Specialist
USDA, SE, ARS, NCR
2000 West Pioneer Parkway
Peoria, IL

THROUGH: Dr. Charles W. Alexander
Area Director
Mid-Great Plains Area
Columbia, MO

Dr. Robert R. Oltjen
Director
Roman L. Hruska U. S. Meat Animal Research Center
Clay Center, NE

Dr. J. Joe Ford
Research Leader
Roman L. Hruska U. S. Meat Animal Research Center
Clay Center, NE

Enclosed please find the Final Report for appraisal and records.

RONALD K. CHRISTENSON
Research Physiologist

Encl.

FINAL REPORT

Specific Cooperative Agreement No. 58-519B-0-899 Between University of Nebraska, Agricultural Experiment Station and SEA, AR.

Title: Investigate the Biological Basis of Sexual Maturation in the Pig.

CRIS No. 3090-20372-016A

1. Description of work results, conclusions and recommendations are included in the copies of the four manuscripts included in this report.
2. Thesis Title
Pubertal Development of the Boar: Age-Related Changes in Testicular Morphology and Selected Endocrine Parameters
Rodney Duane Allrich
1981
University of Nebraska, Lincoln, Nebraska

3. Rodney Duane Allrich

4. *Dwane R. Zimmerman*
Dwane R. Zimmerman

6-26-82
Date



University of
Nebraska
Lincoln

Institute of Agriculture and Natural Resources

Animal Science Department
Lincoln, NE 68583-0908

Phone (402)472-6424



June 28, 1982

Dr. Ron K. Christenson
MARC
P.O. Box 166
Clay Center, NE 68933

Dear Ron:

I want to take this opportunity to express my appreciation to you personally and others on the MARC staff who worked directly with Dr. Allrich on this project to accomplish the objectives we had set out. This study generated some important basic research findings regarding pubertal development in boars and was able to be much more comprehensive because of the excellent laboratory facilities available at Clay Center and the experience and expertise that you and others, e.g., Joe Ford and Don Lunstra at Clay Center provided relative to important approaches and techniques utilized in the study.

I believe Dr. Allrich received an excellent Ph.D. program working with both the University of Nebraska and MARC. I believe it was mutually advantageous to both programs but am certain it was from our standpoint.

I would hope that we might have the opportunity to develop another cooperative agreement involving another Ph.D. student at some point in the future involving a problem that holds the mutual interest and excitement that this one did.

Sincerely yours,

Dwane R. Zimmerman
Professor of Animal Science

DRZ:kp
Enclosures

CURRICULUM VITAE (prepared 4/1/82)

Rodney D. Allrich
2114 Charles St.
Lafayette, Indiana 47904

Telephone: Home (317)742-7582
Office (317)494-4844
Department of Animal Sciences
Lilly Hall
Purdue University
West Lafayette, IN 47907

Personal:

| | |
|---------------------------------------|--------------------|
| Date of Birth: January 21, 1953 | Height: 5'10" |
| Place of Birth: Maddock, North Dakota | Weight: 170 pounds |
| Marital Status: Married, no children | Health: Excellent |

Education:

| | |
|--------------|---|
| 1981-present | Postdoctoral Research Associate, Department of Animal Sciences, Purdue University. Involved in research under project entitled "Neuroendocrine Mechanisms Contributing to Postpartum Anestrus in Beef Cows". |
| 1978-1981 | University of Nebraska, Lincoln, and Roman L. Hruska U.S. Meat Animal Research Center (Ph.D.) Reproductive Physiology/Major; GPA - 3.6/4.0 Ph.D. Dissertation: "Pubertal Development Of The Boar: Age-Related Changes In Testicular Morphology And Selected Endocrine Parameters" |
| 1975-1978 | North Dakota State University, Fargo (M.S.) Reproductive Physiology/Major; GPA - 3.3/4.0 Master's Thesis: "The Effect Of Fasting, Imposed At Weaning, On Various Reproductive Phenomena Of The Gilt" |
| 1971-1975 | North Dakota State University, Fargo (B.S.) Animal Science/Major; GPA - 3.0/4.0 |

Research Experience:

| | |
|-----------------|--|
| Undergraduate | - Executed research project involving Vitamin C and its effect on egg production in laying hens. |
| Graduate (M.S.) | - Enzyme analysis in blood; blood collection from farm species; swine surgery-ovariohysterectomy; RIA-steroids; artificial insemination of bovine and porcine; semen collection and processing; data processing-computer analysis; histology-technique and interpretation. |

- Graduate (Ph.D.) - Laparoscopy technique in swine; RIA of steroids and LH; cytogenetic analysis and interpretation; testicular morphological and morphometry investigation involving fixation and processing for light and electron microscopy; in vitro testicular incubation technique; venous cannulation and castration of boars during all stages of development; miscellaneous surgery techniques; conducted two extensive experiments involving reproductive characterization of three genetic lines of rats.
- Postdoctoral - Dissection of neuroendocrine tissue of the brain; superfusion of bovine pituitary stalk-median eminence; RIA of LHRH; venous cannulation of cattle.

Teaching Experience:

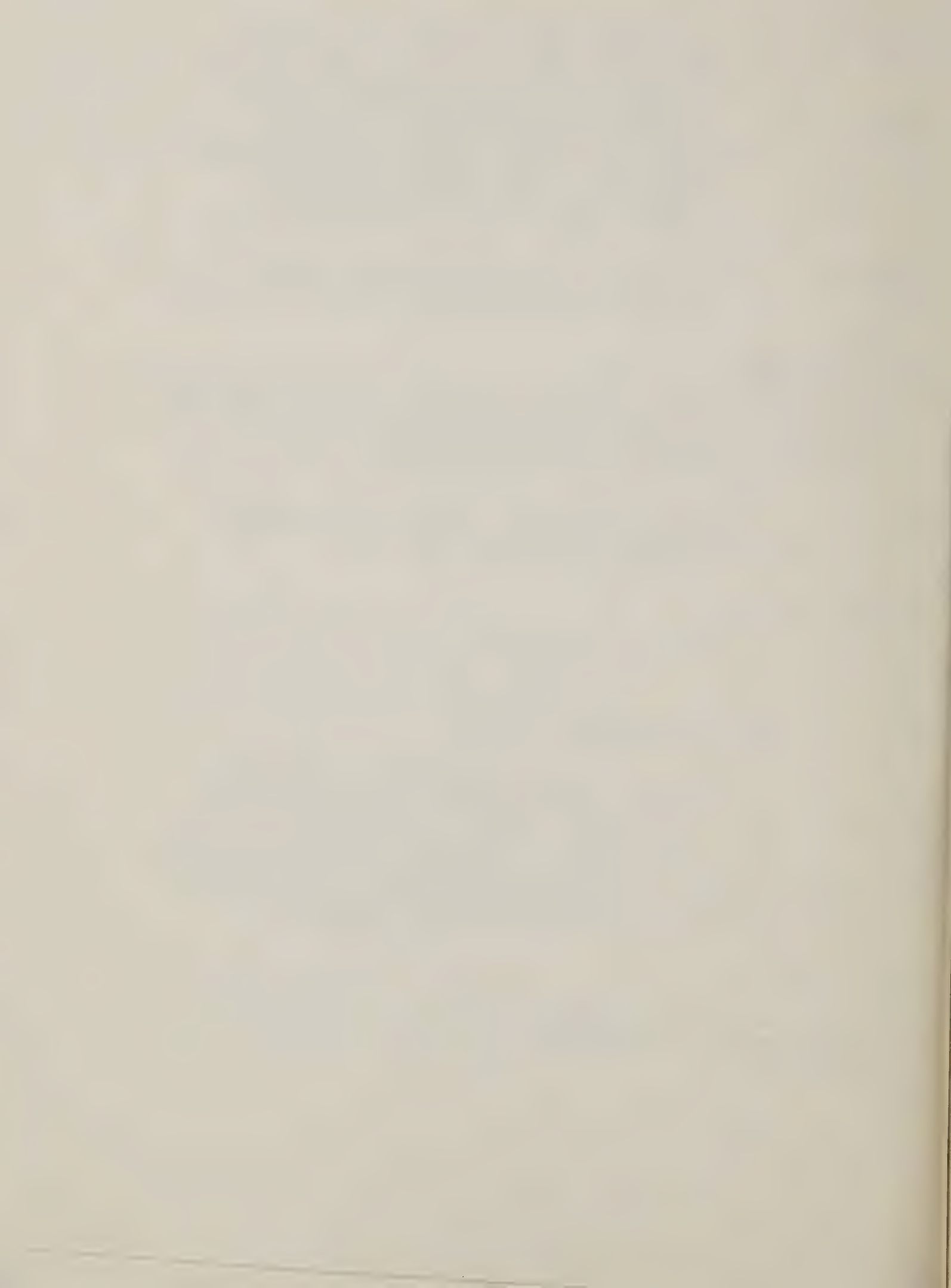
- M.S. - Taught Reproductive Physiology laboratories- involved endocrinology experiments, semen collection and evaluation, gross and microscopic anatomy. Taught "Feeds and Feeding" laboratories - involved mainly balancing rations. Assisted in introductory Animal Science course concerned mainly with livestock judging.
- Ph.D. - Taught introductory Animal Science Laboratory course involved with livestock judging and general Animal Science information. Assisted with Reproductive Physiology laboratories.

Specific Achievements:

- High School - President (two years) and Secretary of local FFA chapter.
- B.S. - Worked way through college, earned 75% of expenses.
- M.S. - Awarded graduate research assistantship. Presented two abstracts at regional meetings.
- Ph.D. - Awarded graduate research assistantship at University of Nebraska with a cooperative research agreement with the Roman L. Hruska U.S. Meat Animal Research Center at Clay Center, Nebraska. Presented five abstracts at regional and national meetings. An additional abstract from the Ph.D. research will be presented in the summer of 1982. Attended the 9th Annual Training Course for RIA sponsored by the Endocrine Society held at Bethesda, Maryland.

Society Memberships:

American Society of Animal Science
Society for the Study of Reproduction
Gamma Sigma Delta



References:

Dr. R. K. Christenson
Roman L. Hruska U.S. Meat
Animal Research Center
P.O. Box 166
Clay Center, NE 68933
(402)762-3241

Dr. Dwane R. Zimmerman
Animal Science Department
Marvel Baker Hall
University of Nebraska
Lincoln, NE 68583
(402)472-6424

Dr. G. E. Moss
Department of Animal Sciences
Purdue University
West Lafayette, IN 47907
(317)494-4813

Dr. J. E. Tilton
Animal Science Department
North Dakota State University
Fargo, North Dakota 58102
(701)237-7641

Publications

Scientific Journal Publications

- Allrich, R. D., J. E. Tilton, J. N. Johnson, W. D. Slanger and M. J. Marchello. 1979. Effect of lactation length and fasting on various reproductive phenomena of sows. J. Anim. Sci. 48:359.
- Allrich, R. D., C. T. Wang, G.E. Dickerson and Dwane R. Zimmerman. 1981. Selection for increased rate or efficiency of lean growth in rats: Correlated responses in reproductive performance. J. Anim. Sci. 53:1458.
- Allrich, R. D., R. K. Christenson, J.J. Ford and Dwane R. Zimmerman. 1982. Pubertal development of the boar: Testosterone, estradiol-17 β , cortisol and LH concentrations before and after castration at various ages. J. Anim. Sci. (in press).
- Allrich, R. D., R. K. Christenson, J. J. Ford and Dwane R. Zimmerman. 1982. Pubertal development of the boar: Age-related changes in testicular morphology and in vitro production of testosterone and estradiol-17 β . Biol. Reprod. (submitted).
- Allrich, R. D., R. K. Christenson and J. J. Ford. 1982. Age at puberty and estrous activity in purebred and reciprocally crossbred gilts. (in preparation).
- Allrich, R. D., S. Melin, R. K. Christenson, J. J. Ford, G. E. Dickerson and Dwane R. Zimmerman. 1982. Effect of crossfostering rats previously selected for postweaning rate and efficiency of lean growth : Reproductive characteristics. (in preparation).

Abstracts

- Allrich, R. D., J. E. Tilton, J. N. Johnson and R. C. Zimprich. 1978. Effect of fasting on various reproductive phenomena of the gilt. J. Anim. Sci. 47 (Suppl. 1):2.
- Allrich, R. D., R. M. Weigl and J. E. Tilton. 1978. Caffeine and its influence on reproduction in the early post-puberal female rat. N. D. Academy Sci. 32:28.
- Allrich, R. D., C. T. Wang, G. E. Dickerson and Dwane R. Zimmerman. 1979. Selection for increased rate or efficiency of lean growth in rats: Correlated responses in reproductive performance. J. Anim. Sci. 49 (Suppl. 1):278.
- Allrich, R. D., Dwane R. Zimmerman and G. E. Dickerson. 1980. Effect of crossfostering rats previously selected for rate or efficiency of lean growth: Preputial separation, body and testes weight responses. J. Anim. Sci. 51 (Suppl. 1):86.
- Allrich, R., G. E. Dickerson, D. R. Zimmerman and C. T. Wang. 1980. Maternal and individual preweaning effects of selection for postweaning rate and efficiency of lean growth in rats. J. Anim. Sci. 51 (Suppl. 1):111.
- Wise, M., A. Jones, R. Allrich and Dwane R. Zimmerman. 1980. Influence of photoperiod and relocation-boar exposure stimuli on age at puberty in confinement-reared gilts. J. Anim. Sci. 51 (Suppl. 1):88.
- Zimmerman, Dwane R., M. Wise, A. P. K. Jones, R. D. Allrich and R. K. Johnson. 1980. Testicular growth in swine as influenced by photoperiod (16L-8D vs 8L-16D) and ovulation rate selection in females. J. Anim. Sci. 51 (Suppl. 1):340.
- Cleveland, E. R., C. T. Wang, R. Allrich, W. R. Lamberson and G. E. Dickerson. 1980. Maternal efficiency of rats selected for rapid or for efficient postweaning lean growth. J. Anim. Sci. 51 (Suppl. 1):114.
- Allrich, R. D., R. K. Christenson and J. J. Ford. 1981. Age at puberty and estrous activity in purebred and reciprocally crossbred gilts. J. Anim. Sci. 53 (Suppl. 1):104.
- Allrich, R. D., R. K. Christenson, J. J. Ford and Dwane R. Zimmerman. 1981. Pubertal development of boars: LH, testosterone and estradiol-17 β concentrations before and LH concentrations after castration at various ages. J. Anim. Sci. 53 (Suppl. 1):292.
- Allrich, R. D. and R. K. Christenson. 1981. Age differences in the response to hCG by porcine testicular tissue in vitro. Biol. Reprod. 24 (Suppl. 1):132A.

Abstracts (con't)

- Wise, M. E., R. D. Allrich, A. Jones, R.J. Kittok and Dwane R. Zimmerman. 1981. Influence of photoperiod (16L:8D vs 8L:16D) and size of rearing group (10 vs 30) on age at puberty in confinement-reared gilts. J. Anim. Sci. 53 (Suppl. 1):104.
- Allrich, R. D., R.K. Christenson and Dwane R. Zimmerman. 1982. Pubertal development of the boar: Age-related changes in testicular in vitro estradiol-17 β production. Biol. Reprod. 26 (Suppl. 1):000.
- Allrich, R. D., G. E. Moss and P. V. Malven. 1982. In vitro release of LH-releasing hormone (LHRH) from superfused tissue of the bovine pituitary stalk-median eminence. J. Anim. Sci. 55 (Suppl. 1):000.
- Berardinelli, J. G., R. D. Allrich and J. J. Ford. 1982. Characterization of LH-hCG receptor in testis of boars and its relationships with morphological and steroidogenic characteristics during development. J. Anim. Sci. 55 (Suppl. 1):000.

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Authors show first (1) and second (2) choices of sessions in which they would like their paper scheduled:

- ☐ Animal Behavior
- ☒ Animal Breeding and Genetics
- ☐ Animal Waste Management
- ☐ Environment and Livestock Production
- ☐ Meat Science and Technology
- ☐ Non-ruminant Nutrition
- ☐ Pastures and Forages
- ☐ Physiology
- ☐ Ruminant Nutrition
- ☐ Teaching
- ☐ Extension

Corresponding author R.D. Allrich

Address Department of Animal Science

University of Nebraska

Lincoln, NE 68583

List key words at end of abstract inside blue lines.

ABSTRACT AND ABSTRACT HEADING (see example, end of instructions)

☒ Selection for increased rate or efficiency of lean growth in rats: Correlated responses in reproductive performance. R.D. Allrich*, C.T. Wang, G.E. Dickerson and Dwane R. Zimmerman, University of Nebraska-Lincoln.

Reproductive performance was evaluated in two select lines (LG, selected for postweaning lean growth; ELG, selected for efficiency of postweaning lean growth) and a randomly selected control line (C) to determine if any changes in reproductive performance had accompanied the genetic improvement in rate and efficiency of lean growth after 14 generations of selection. All progeny (179 males and 402 females) were reared in standardized litters of nine until weaning at 21 days of age and in like sex (LS) or unlike sex (US) pairs thereafter. LS paired females were paired with similar aged males for mating at approximately 70 days of age while US paired females remained with the original male until mated or autopsied at 85 days of age. Parameters measured for females included age (AVO) and weight (WVO) at vaginal opening, ovulation rate (OR), number of fetuses (F), pre- and post-implantation loss (PRIL and PIL, respectively) measured at 16-18 days of pregnancy and number of pups born (NB). Males were evaluated for age (AM) and weight (WM) at first spontaneous mating and testis weight (TW) and body weight (BW) at 85 days. Selection for either LG or ELG increased AVO (38.9 and 38.6 vs 36.9 d for C, $P < .01$) and WVO (133.1 and 126.6 vs 119.8 g for C, $P < .05$) in LS paired females. In US paired females, AVO was delayed in the LG line (39.7 vs 37.0 d for C, $P < .05$) but not in the ELG line (38.3 d) while WVO was increased for both LG and ELG lines (135.6 and 133.3 vs 120.1 g for C, $P < .05$). OR, F, PRIL, PIL and NB were not altered by selection for LG or ELG. AM did not differ among lines but LG males were heavier at mating than controls (389 vs 294 g for C, $P < .05$). TW was lowered in males selected for ELG as compared to controls (3.35 vs 3.54 g for C, $P < .05$). BW at 85 days was increased in LG males as compared to controls (428 vs 381 g for C, $P < .01$).

KEY WORDS: Lean Growth, Selection, Reproductive Traits, Rats

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13th ANNUAL MEETING MIDWESTERN SECTION
OF THE AMERICAN SOCIETY OF ANIMAL SCIENCE

June 11-13, 1980 — Manhattan, Kansas

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Authors show first (1), second (2), and third (3) choices:

- ☒ 1 Breeding and Genetics
- ☒ 2 Environment and Livestock Production
- ☐ 3 Extension
- ☐ Meat Science and Muscle Biology
- ☐ Non-Ruminant Nutrition
- ☐ Pastures and Forages
- ☒ 4 Physiology
- ☐ Ruminant Nutrition
- ☐ Teaching

EXAMPLE OF ABSTRACT HEADING AND ABSTRACT

| | |
|-------------------------------------|--|
| <input checked="" type="checkbox"/> | Fertility of beef females followed controlled estrous cycles and ovulation. A. A. Zaid*, W. D. Humphrey, C. C. Kaltenbach, and T. G. Dunn, University of Wyoming, Laramie. |
| | Pregnancy rates (PR) following two progestogen implant periods and breeding at either controlled ovulation or 12 hr after synchronized estrus were compared. . . |

List key words at end of abstract inside blue lines.

ABSTRACT AND ABSTRACT HEADING (see example)

Effect of crossfostering rats previously selected for rate or efficiency of lean growth: Preputial separation, body and testes weight responses. R.D. Allrich*, Dwane R. Zimmerman and G.E. Dickerson, University of Nebraska-Lincoln and USDA, SEA, AR, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE.

Preputial separation and testes weight at 85 days of age were evaluated in a crossfostering experiment utilizing three lines of rats (LG, selected for rate of post-weaning lean growth; LE, selected for efficiency of postweaning lean growth; C, randomly selected control line). Significant increases have occurred in the selected parameters. Dams were bred to unrelated males within the same genetic line. Within 12 hr of birth pups were weighed, toe notched and crossfostered. Six female and three male pups were used from each litter. Dams reared one male and two female pups from her litter and the same number of pups from a dam of each of the other two lines. A total of 360 male pups were weaned at 21 days and thereafter housed in groups of two. Age (APS) and body weight (WPS) at preputial separation and body (BW) and testes (TW) weight at 85 days were recorded. LE males had greater ($P < .01$) APS than LG and C males (52.1 vs 50.3 and 49.2 days, respectively). APS was decreased ($P < .05$) for LE males reared by C dams and conversely APS was increased ($P < .05$) for C males reared by LE dams. LG males had heavier ($P < .001$) WPS when compared to LE and C males (244.9, 225.8 and 217.9 g, respectively). LG males were heavier (BW, $P < .025$) at 85 days than either LE or C males (415, 386 and 372 g, respectively). LE males had lighter ($P < .01$) testes than LG and C males (3.43 vs 3.74 and 3.74 g, respectively). Crossfostering did not alter BW or TW. Data indicate that APS and WPS are altered by selection for LG and LE and that the postnatal maternal environment can influence APS independent of WPS. BW and TW at 85 days are altered by selecting for LG or LE whereas they are not changed by crossfoster-

KEY WORDS: Crossfostering, Preputial Separation, Testes Weight, Lean Growth, Rate

Must be postmarked before January 15 and should be edited, ready for the printer. All data must be reported in the metric system. If withdrawal of a paper becomes necessary, the Secretary should be notified at once. Mail six copies to: W. R. Woods, Animal Science Department, 3-115 Lilly Hall, Purdue University, West Lafayette, IN 47907 (317-494-6187).

1980 72nd ANNUAL MEETING OF THE
AMERICAN SOCIETY OF ANIMAL SCIENCE
July 27-30, 1980, Cornell University, Ithaca, NY

Last name of first author

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Authors show first and second choices of sessions by marking
number 1 and 2 in the appropriate boxes.

Animal Behavior
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Animal Waste Management
Environment and Livestock Production
Meat Science and Technology
Non-ruminant Nutrition
Pastures and Forages
Physiology
Ruminant Nutrition
Teaching
Extension

Corresponding author G. E. Dickerson

Address 225 Marvel Baker Hall

University of Nebraska

Lincoln, Nebraska 68583

List key words at end of abstract inside blue lines.

Would you be willing to present this paper in
poster session? Yes ☒ No

ABSTRACT AND ABSTRACT HEADING (see example, end of instructions)

☒ Maternal and individual preweaning effects of selection for postweaning rate and efficiency of lean gain in rats. R. Allrich, G. E. Dickerson*, D. R. Zimmerman and T. Wang, University of Nebraska, Lincoln and R. L. Hruska U.S. Meat Animal Research Center, AR-SEA, USDA, Clay Center, Nebraska

cross-fostering design was used to measure cumulative effects of 14 generations of selection for rate (LG) and efficiency (LE) of postweaning lean gain on maternal and individual genetic components of preweaning growth and mortality, relative to unselected controls (C). In the 8th generation of relaxed selection, 120 pair-matings were made to obtain 90 vaginal-plug monitored pregnancies per line. From these, 40 sets were obtained of one litter from each LG, LE and C lines born on same day. Each female in a set nursed three of her own pups and three from each of the other two litters to 21 days of age. Nurse effects of LE were below C for pup survival by -4% at 12 days and -6% at 21 days and those of LG were above C for pup weights by 4%*** at 12 days and 3% at 21 days, with total litter weights at 1 days +2% for LG and -3% for LE females. Pup genotype for survival was +2% for LG and 2% for LE at 12 days, +1% for LG and -3% for LE at 21 days; for pup weight was +4%* for LG and -1% for LE at 12 days and +1% for LG and -6%* for LE at 21 days and for total litter weight at 21 days +2% for LG and -9% for LE. Interactions of nurse line and pup genotype, including fostered vs non-fostered, were negligible. LG selection improved both maternal and pup genotype effects on preweaning growth, whereas LE selection reduced maternal effects on survival and pup genotype effects on both survival and growth to weaning. However, LG females were larger than LE or C females and had larger total feed intake requirements for both maintenance and lactation.

KEYWORDS: Rats, Selection, Lean Growth, Maternal Effects

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Animal Behavior
Animal Breeding and Genetics
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Environment and Livestock Production
Meat Science and Technology
Non-ruminant Nutrition
Pastures and Forages
Physiology
Ruminant Nutrition
Teaching
Extension

Corresponding author G. E. Dickerson

Address 225 Marvel Baker Hall

University of Nebraska

Lincoln, Nebraska 68583

List key words at end of abstract inside blue lines.

Would you be willing to present this paper in
poster session? Yes ☒ No

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Cross-fostering design was used to measure cumulative effects of 14 generations of selection for rate (LG) and efficiency (LE) of postweaning lean gain on maternal and individual genetic components of preweaning growth and mortality, relative to unselected controls (C). In the 8th generation of relaxed selection, 120 pair-matings were made to obtain 90 vaginal-lug monitored pregnancies per line. From these, 40 sets were obtained of one litter from each LG, LE and C lines born on same day. Each female in a set nursed three of her own pups and three from each of the other two litters to 21 days of age. Nurse effects of LE were below C for pup survival by -4% at 12 days and -6%+ at 21 days and those of LG were above C for pup weights by 4%*** at 12 days and 3% at 21 days, with total litter weights at 1 day +2% for LG and -3% for LE females. Pup genotype for survival was +2% for LG and -2% for LE at 12 days, +1% for LG and -3% for LE at 21 days; for pup weight was +4%* for LG and -1% for LE at 12 days and +1% for LG and -6%* for LE at 21 days and for total litter weight at 21 days +2% for LG and -9% for LE. Interactions of nurse line and pup genotype, including fostered vs non-fostered, were negligible. LG selection improved both maternal and pup genotype effects on preweaning growth, whereas LE selection reduced maternal effects on survival and pup genotype effects on both survival and growth to weaning. However, LG females were larger than LE or C females and had larger total feed intake requirements for both maintenance and lactation.

KEY WORDS: Rats, Selection, Lean Growth, Maternal Effects

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Authors show first (1), second (2), and third (3) choices:

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- ☒ Physiology
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- ☐ Teaching

EXAMPLE OF ABSTRACT HEADING AND ABSTRACT

| | |
|-------------------------------------|---|
| <input checked="" type="checkbox"/> | Fertility of beef females followed controlled estrous cycles and ovulation. A. A. Zaied*, W. D. Humphrey, C. C. Kaltenbach, and T. G. Dunn, University of Wyoming, Laramie. |
| | Pregnancy rates (PR) following two progestogen implant periods and breeding at either controlled ovulation or 12 hr after synchronized estrus were compared. . . |

List key words at end of abstract inside blue lines.

ABSTRACT AND ABSTRACT HEADING (see example)

☒ Influence of photoperiod and relocation-boar exposure stimuli on age at puberty in confinement-reared gilts. M. Wise*, A. Jones, R. Allrich and Dwane R. Zimmerman, University of Nebraska-Lincoln.

The influences of photoperiod and relocation-boar exposure stimuli on age at puberty were evaluated in 170 August-September born gilts from sows selected for high ovulation rate (Select line) and randomly selected control line sows. Gilts were assigned at random to two photoperiod regimens, 8 hr light:16 hr darkness (8L-16D) and 16 hr light:8hr darkness (16L-8D), at 3 days of age. Light intensity ranged from 150-200 lux at eye level throughout development. Gilts were moved from nursery to a totally enclosed development house at 70 days of age and allocated according to birth date to three blocks of pens in each light treatment (room). At 165 days of age, litters within room and block were stratified at random between three treatments: regrouped but maintained in the same room and block from day 70 (Control); regrouped, relocated to a different room (same photoperiod) and provided once daily contact with a mature boar (R & BE-165; same as R & BE-165 except relocation and boar exposure delayed until 185 days (R & BE-185). Gilts were observed daily for symptoms of estrus without the aid of a boar from 140 days of age until 165 days (R & BE-165), 185 days (R & BE-185), or until termination of the experiment at 257 days of age (Control). Gilts were laparoscoped or slaughtered to confirm ovulation at first estrus. Age at puberty in gilts that expressed estrus did not differ between 16L-8D and 8L-16D photoperiod regimens (208.3 vs 204.7 days, respectively). However, gilts relocated and exposed to a boar attained puberty 24.1 days earlier than unstimulated controls (R & BE-165 and R & BE-185, 197 days vs Control, 221.9 days, $P < .01$). Gilts subjected to relocation-boar exposure stimuli at 165 days expressed puberty 18.6 days earlier than gilts provided these stimuli at 185 days of age (R & BE-165, 183.5 days vs R & BE-185, 207.1 days, $P < .01$). The percentage of gilts not cycling by 257 days was 11.6 for control, 3.6 for R & BE-165 and 5.1 for R & BE-185. No difference in age at puberty was detected between control and select line gilts.

KEY WORDS: Puberty, Photoperiod, Swine, Boar Exposure

1980 72nd ANNUAL MEETING OF THE AMERICAN SOCIETY OF ANIMAL SCIENCE

July 27-30, 1980, Cornell University, Ithaca, NY

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Teaching
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Corresponding author Dwane R. Zimmerman

Address Animal Science Department

University of Nebraska

Lincoln, NE 68583

List key words at end of abstract inside blue lines.

Would you be willing to present this paper in
poster session? Yes X No

ABSTRACT AND ABSTRACT HEADING (see example, end of instructions)

☒ Testicular growth in swine as influenced by photoperiod (16L-8D vs 8L-16D) and ovulation rate selection in females. Dwane R. Zimmerman*, M. Wise, A. P. K. Jones, R. D. Allrich and R. K. Johnson, University of Nebraska, Lincoln

Two trials were conducted to evaluate the influence of photoperiod and ovulation rate selection in females on testicular growth in males. Two photoperiod regimes (16L-8D, 16 hr light and 8 hr dark vs 8L-16D, 8 hr light and 16 hr dark) and two genetic lines (C, randomly selected control line vs S, females mass selected for high ovulation rate) were compared in each trial. Select boars in trials 1 and 2 represented the 1st and 2nd generations of relaxed selection following 9 generations of effective selection for high ovulation rate. Equal numbers of boars from each line (trial 1, 54 C and 54 S, born Aug.-Sept., 1978; trial 2, 34 C and 24 S, born Feb.-Mar., 1979) were randomly allotted to each photoperiod regimen (3 days, trial 1: 28 days, trial 2) and to three castration groups (140, 182 and 224 days of age). Light was produced with fluorescent lamps (cool white). The intensity ranged from 150-200 lux at eye level during development. Body weights (BW) were recorded at frequent intervals and on the day the testes were removed. Each testis was identified, placed on ice and dissected free of the epididymis and extraneous tissue before recording weight (TW). The data from each trial were pooled for analysis of variance since none of the interactions between main effects was significant. Photoperiod failed to influence either BW (140, 79 vs 79 kg; 182, 107 vs 110 kg; 224, 122 vs 134 kg for 16L-8D and 8L-16D, respectively) or TW (140, 118 vs 115 g; 182, 241 vs 233 g; 224, 267 vs 276 g for 16L-8D and 8L-16D, respectively) at any stage of castration. Select line boars, however, produced heavier testes weights than controls at all ages (140, 134 vs 104; 182, 253 vs 226; 224, 284 vs 263, $P < .01$).

KEY WORDS: Photoperiod, Testes, Swine, Selection, Ovulation Rate.

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1980 72nd ANNUAL MEETING OF THE
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Corresponding author Erik R. Cleveland

Address 230 Marvel Baker Hall

University of Nebraska

Lincoln, Nebraska 68583

List key words at end of abstract inside blue lines.

Would you be willing to present this paper in poster session? Yes ☒ No

ABSTRACT AND ABSTRACT HEADING (see example, end of instructions)

☒ Maternal efficiency of rats selected for rapid or for efficient postweaning lean growth. E. R. Cleveland*, C. T. Wang, R. Allrich, H. R. Lamberson and J. E. Dickerson, University of Nebraska, Lincoln and R. L. Hruska U.S. Meat Animal Research Center, AR-SEA, USDA, Clay Center, Nebraska

Effects on maternal efficiency from 14 generations of between family selection for rapid (LG) or for efficient (LE) postweaning lean growth were evaluated relative to unselected controls (C) after 8 generations of relaxed selection, from feed consumption, body weights and preweaning litter mortality and weights for 40 balanced sets of 3 contemporary cross-fostered litters (3 LG, 3 LE, 3 C pups/female). LG exceeded LE and C females in weight at conception (295, 275 and 272 g), at gestation day 14 (350, 329 and 322 g) and 20 (446, 404 and 403 g), after parturition (337, 307 and 310 g), at lactation day 12 (348, 323 and 324 g), at 21-day weaning (315, 304 and 303 g) and at 10 days after weaning (333, 309 and 307 g). The slightly lower number and total weight of pups weaned by LE females (7.95 vs 8.4 and 8.5 pups for LG and C; 305 vs 323 and 316 g) and smaller weight loss of LE females during lactation (3 vs 22 and 7 g) suggest lower milk production by LE females. Feed consumption was higher for LG than for LE or C females for 0-14 days gestation (304 vs 294 and 87 g) and especially for 0-21 days lactation (972 vs 872 and 902 g) and 10 days after weaning (307 vs 266 and 268 g). The total feed intake from conception to 10 days after weaning exceeded C by 7% for LG and 2% for LE per gram of litter weaned and by 10% for LG and 5% for LE per pup weaned. Thus, increased efficiency of postweaning gain for LG and LE over C rats was partly offset by higher maternal overhead costs, especially from the larger size LG females and the higher preweaning mortality of pups nursed by LE females.

KEY WORDS: Rats, Selection, Lean Growth, Maternal Efficiency

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14th ANNUAL MEETING MIDWESTERN SECTION
OF THE AMERICAN SOCIETY OF ANIMAL SCIENCE

June 9-10, 1981 - Lincoln, Nebraska

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EXAMPLE OF ABSTRACT HEADING AND ABSTRACT

| | |
|-------------------------------------|---|
| <input checked="" type="checkbox"/> | Fertility of beef females following controlled estrus cycles and ovulation. A. A. Zaied*, W. D. Humphrey, C. C. Kaltenbach, and T. G. Dunn, University of Wyoming, Laramie. |
| | Pregnancy rates (PR) following two progestogen implant periods and breeding at either controlled ovulation or 12 hr after synchronized estrus were compared. . . |

List key words at end of abstract inside blue lines.

ABSTRACT AND ABSTRACT HEADING (see example)

Age at puberty and estrous activity in purebred and reciprocally crossbred gilts.
R. D. Allrich*, R. K. Christenson and J. J. Ford, University of Nebraska, Lincoln
and USDA, SEA-AR, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE.

Data from Landrace (LR) and Yorkshire (Y) purebred (LRxLR, YxY) and reciprocally crossbred (LRxY, YxLR) gilts were analyzed for age at puberty (AP) and percent showing regular estrous cycles (REC) at 6.5, 7.5 and 8.5 months of age. Trial I was comprised of gilts from all breed combinations while Trial II included only reciprocally crossbred gilts. Gilts were reared in total confinement in groups of 10 to 12 with ad libitum feeding until gilts reached 100 kg whereafter they received 1.82 kg feed per head daily. Daily estrous checks were performed from 4.5 to 8.5 months of age whereupon reproductive tracts from gilts not showing REC were examined by laparoscopy to determine reproductive status. In comparisons (Trial I) of YxY to LRxLR, YxLR and LRxY gilts, respectively, the following results were obtained: YxY gilts reached puberty at an older ($P<.05$) age (199.8 vs 184.7, 186.0 and 187.2 days), had the lowest ($P<.01$) percentage showing REC at 6.5 months (38 vs 72, 68 and 74), had a similar percentage showing REC at 8.5 months (76, 90, 77 and 89), and had the highest ($P<.05$) percent prepubertal at 8.5 months (17 vs 3.5, 0 and 0). Percent behaviorally anestrous at 8.5 months did not differ (3.5, 0, 9.7 and 7.4). In Trial II, reciprocally crossbred YxLR and LRxY gilts did not differ for age at puberty (183.6 and 180.0 days), percentage showing REC at 6.5 months (62 and 71), at 8.5 months (79 and 82), percent prepubertal at 8.5 months (4.8 and 5.3) and percent behaviorally anestrous at 8.5 months (14.3 and 13.2). In both trials reciprocally crossbred gilts did not differ in AP and percentage showing REC, while AP failed to exhibit any significant heterosis effect.

KEY WORDS: Reciprocally Crossbred Gilts, Puberty, Estrous Activity

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1981 73rd ANNUAL MEETING OF THE AMERICAN SOCIETY OF ANIMAL SCIENCE

Last name of first author

July 26-29, 1981, North Carolina State University, Raleigh

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Corresponding author R. D. AllrichAddress Roman L. Hruska U.S. Meat Animal
Research Center
P.O. Box 166
Clay Center, NE 68933
Phone 402/762-3241PAPER EVALUATION (indicate if you wish to have your
oral presentation evaluated):

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ABSTRACT AND ABSTRACT HEADING (see example, end of instructions)

~~X~~ Pubertal development of boars: LH, testosterone and estradiol-17 β concentrations before and LH concentrations after castration at various ages. R. D. Allrich^{1,2*}, K. Christenson², J. J. Ford², and Dwane R. Zimmerman¹, ¹Department of Animal Science, University of Nebraska, Lincoln and ²USDA, SEA-AR, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE

Forty-eight Landrace x Duroc boars were assigned at weaning to one of eight castration ages (40, 70, 100, 130, 160, 190, 220 and 250 days). Catheterization of the jugular was performed five days (-5) before castration. Blood samples were taken every ½ hr between 0800 and 1200 two days (-2) before and on days +1, +2, +3, +4, +8 and +16 after castration. Serum concentrations of LH, testosterone (T) and estradiol-17 β (E₂) were quantified by RIA on pooled (within boar) samples. Mean concentrations of T and E₂ (-2 day samples) increased linearly (P<.01) with age of boar (0.9, 1.8, 4.0, 8.5, 8.5, 14.2, 14.3 and 14.9 ng T/ml and 11, 19, 16, 53, 89, 98, 108 and 118 pg E₂/ml at 40, 70, 100, 130, 160, 190, 220 and 250 days, respectively).

Mean LH concentrations before and after castration are given in the table. LH concentrations were elevated within 1 to 2 days following castration at 40, 70, 100, 130 and 160 days. These data suggest that the negative feedback influence of testicular steroids on LH secretion is diminished after 160 days in the boar.

| Age (days) | LH (ng/ml) | | |
|---------------|---------------------|--------|--------|
| | Day from castration | | |
| | -2 | +1 | +2 |
| 40 | 0.83 | 1.13* | 1.33** |
| 70 | 1.08 | 1.32 | 1.40* |
| 100 | 0.88 | 1.50** | 1.17* |
| 130 | 1.11 | 1.58** | 1.38* |
| 160 | 1.17 | 1.48* | 1.58** |
| 190 | 1.13 | 1.27 | 1.32 |
| 220 | 1.02 | 1.23 | 1.17 |
| 250 | 1.25 | 1.50 | 1.42 |

*P<.05, **P<.01 (Compared with day -2).

KEY WORDS: Boar, Puberty, LH,
Testosterone, Estradiol, Castration

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SOCIETY FOR THE STUDY OF THE REPRODUCTION

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ON OR BEFORE FEBRUARY 15

AGE DIFFERENCES IN THE RESPONSE TO HCG BY PORCINE TESTICULAR TISSUE IN VITRO. R. D. Allrich¹* and R. K. Christenson², ¹Department of Animal Science, University of Nebraska, Lincoln and ²USDA, SEA-AR, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE.

Events initiating changes in testis function of the pubertal boar are largely unknown. The purpose of the present study was to investigate the response of testicular tissue, obtained from 42 crossbred boars at 40, 70, 100, 130, 160, 190 and 220 days of age, to hCG stimulation in vitro. At castration, 500 mg pieces of testicular tissue were minced in 5 ml of incubation media (TC 199). After a one-half h preincubation period the media was exchanged with media containing either 0, 5, 25, 125, 625 or 3125 mIU hCG/ml. A time-course study of in vitro testosterone (T) production was then conducted over a three h period at 36 C. T concentrations in the media were quantified by RIA. Results obtained after 1 h incubation were:

Testosterone production, ng/mg protein

| mIU/ml | Boar age (days) | | | | | |
|--------|-----------------|-------|-------|-------|-------|-------|
| | 40 | 70 | 100 | 130 | 160 | 190 |
| 0 | 4.36 | 8.20 | 11.50 | 15.63 | 8.76 | 8.28 |
| 5 | 11.53 | 14.24 | 17.25 | 38.28 | 13.36 | 12.50 |
| 25 | 16.96 | 20.27 | 23.00 | 53.13 | 18.39 | 14.00 |
| 125 | 25.48 | 26.08 | 32.45 | 63.59 | 22.99 | 18.98 |
| 625 | --- | 37.36 | 46.61 | 77.66 | 28.45 | 21.23 |
| 3125 | 31.10 | 46.81 | 59.73 | 83.43 | 34.91 | 23.49 |
| | | | | | | 39.57 |

In addition to the dose-dependent increase ($P < .01$) in T production shown, the tissue also displayed a time-dependent increase ($P < .01$) for at least 1 h. Testicular tissue from boars at 130 days of age demonstrated increased ($P < .01$) T production for all hCG dosages evaluated compared to other age groups while morphological examination of fixed testicular tissue from the same boars indicated that the increased T production cannot be explained by increased volume percentage of Leydig cells. It appears that by 130 days the boar testis reaches a critical stage in its development relative to its potential to secrete T.

Name and address of presenting author.

R. D. Allrich

Roman L. Hruska U.S. Meat
Animal Research Center

P.O. Box 166, Clay Center, NE

MAIL TO:

Claude Cruse, Business Manager

Society for the Study of Reproduction

309 West Clark Street

Champaign, IL 61820

14th ANNUAL MEETING MIDWESTERN SECTION
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June 9-10, 1981 - Lincoln, Nebraska

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EXAMPLE OF ABSTRACT HEADING AND ABSTRACT

☒ Fertility of beef females following controlled estrus cycles and ovulation. A. A. Zaied*, W. D. Humphrey, C. C. Kaltenbach, and T. G. Dunn, University of Wyoming, Laramie.

Pregnancy rates (PR) following two progestogen implant periods and breeding at either controlled ovulation or 12 hr after synchronized estrus were compared. . .

List key words at end of abstract inside blue lines.

ABSTRACT AND ABSTRACT HEADING (see example)

☒ Influence of photoperiod (16L:8D vs 8L:16D) and size of rearing group (10 vs 30) on age at puberty in confinement-reared gilts. M.E. Wise*, R.D. Allrich, A. Jones, R.J. Kittok and Dwane R. Zimmerman, University of Nebraska, Lincoln.

The influence of photoperiod and number of gilts reared per pen on age at puberty were evaluated in 153 February-March born gilts from the University synthetic gene pool population. Litters were divided into two replicate groups according to birth date and gilts in each replicate randomly assigned within litter to two photoperiod regimens (8 hr light:16 hr darkness, 8L:16D and 16 hr light:8 hr darkness, 16L:8D) and two rearing group situations (group of 30, G-30, vs group of 10, G-10, gilts per pen). Photoperiod treatment (cool white fluorescent, 100-150 lux) was started immediately following weaning at 4 wk whereas the group size variable was imposed following regrouping and relocation of the gilts from the nursery to a totally enclosed development house at 10 wk. Floor space (4.1 sq. m per gilt) was comparable for the G-10 and G-30 groups. Daily observations for symptoms of estrus were started at 40 days without the aid of a boar. Gilts were relocated to a different room (photoperiod and group situation remained unchanged) and provided once daily contact with a mature boar starting at 165 days of age. Gilts were laparoscoped following first estrus to confirm first ovulation. Estrous cycles were followed on all gilts until termination of the experiment at approximately 255 days of age. Reproductive tracts were recovered at slaughter to determine ovulation rate at the last estrus in cyclic gilts and to confirm prepubertal status of gilts not detected in estrus. Age at puberty was not influenced by photoperiod (8L:16D, 191.3 da vs 16L:8D, 194.9 da, $P < .1$) or by size of the rearing group (G-10, 189.0 da vs G-30, 194.6 da, $P < .1$). Photoperiod and size of the group also failed to influence ovulation rate (8L:16D, 12.6 vs 16L:8D, 12.2 and G-10, 12.9 vs G-30, 12.2). Interval weight gains taken from 28 to 240 days of age showed no influence of photoperiod or rearing group.

KEY WORDS: puberty, photoperiod, group-size, swine

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SOCIETY FOR THE STUDY OF REPRODUCTION

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PUBERTAL DEVELOPMENT OF THE BOAR: AGE-RELATED CHANGES IN TESTICULAR IN VITRO
ESTRADIOL-17 β PRODUCTION. R. D. Allrich^{1,2,*}, R. K. Christenson² and Dwane
R. Zimmerman¹, ¹Department of Animal Science, University of Nebraska, Lincoln and
²USDA, AR, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE.

During pubertal development of the boar, testis size is greatly increased due to growth of the seminiferous tubules and increase in total number of Leydig cells.

The consequences of these alterations on testicular steroid production are largely unknown. The purpose of the present study was to investigate the response of tes-

known, the purpose of the present study was to determine the effect of age and sex on the morphometric characteristics of the parathyroid glands in the parathyroid tissue, obtained from 48 crossbred boars at 40, 70, 100, 130, 160, 190, 220 and 250 days of age to bCC stimulation *in vitro*. In addition, a morphometric

study of the tissue was conducted. At castration, 500 mg pieces of testicular tissue were minced in 5 ml of incubation media (TC 199). After a one-half h preincuba-

sue were minced in 5 ml of incubation media (16 1977). After a one hour preincubation period the media was exchanged with media containing either 0, 5, 25, 125, 250 or 500 ng/ml A time course study of *in vitro* estradiol-17 β (E_2) production by LCC/m-1.

tion was then conducted over a three h period at 36 C. E₂ concentrations in the

media were quantified by KLA. Results obtained after 3 h incubation were:

Estradiol-17 β production, ng/500 mg testis

- Estradiol-17
- β
- production, ng/500 mg testis

Boar age (days)

| hCG mIU/ml | Boar age (days) | | | | | | | |
|---------------|-----------------|-------|-------|--------|-------|-------|-------|------|
| | 40 | 70 | 100 | 130 | 160 | 190 | 220 | 250 |
| 0 | 7.18 | 14.02 | 12.00 | 22.08 | 17.59 | 13.91 | 12.58 | 14.5 |
| 5 | 7.73 | 17.09 | 16.55 | 35.03 | 25.07 | 17.19 | 17.71 | 16.2 |
| 25 | 8.85 | 23.29 | 17.83 | 46.92 | 31.88 | 20.79 | 19.34 | 18.6 |
| 125 | 12.23 | 31.03 | 21.43 | 71.84 | 40.78 | 25.78 | 23.83 | 22.9 |
| 625 | --- | 40.52 | 30.80 | 89.64 | 51.85 | 30.71 | 26.23 | 27.3 |
| 3125 | 22.70 | 47.93 | 45.93 | 102.50 | 62.25 | 37.73 | 27.61 | 29.3 |

E_2 production increased ($P<.01$) in a dose- and time-dependent manner during the entire incubation period. Testicular tissue from boars at 130 days of age demonstrated increased ($P<.05$) E_2 production at all hCG dosages relative to other age groups. No clear relationship could be detected between E_2 production and testicular composition. Taken together, the data indicate that by 130 days of age the boar testis reaches a critical stage in its development relative to E_2 production.

MAIL TO:

Claude Cruse, Executive Secretary

Society for the Study of Reproduction

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Champaign, IL 61820

**1982 JOINT ANNUAL MEETING OF THE
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August 8 - 11, 1982, University of Guelph, Guelph, Ontario, Canada

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EVALUATION (indicate if you wish to have your

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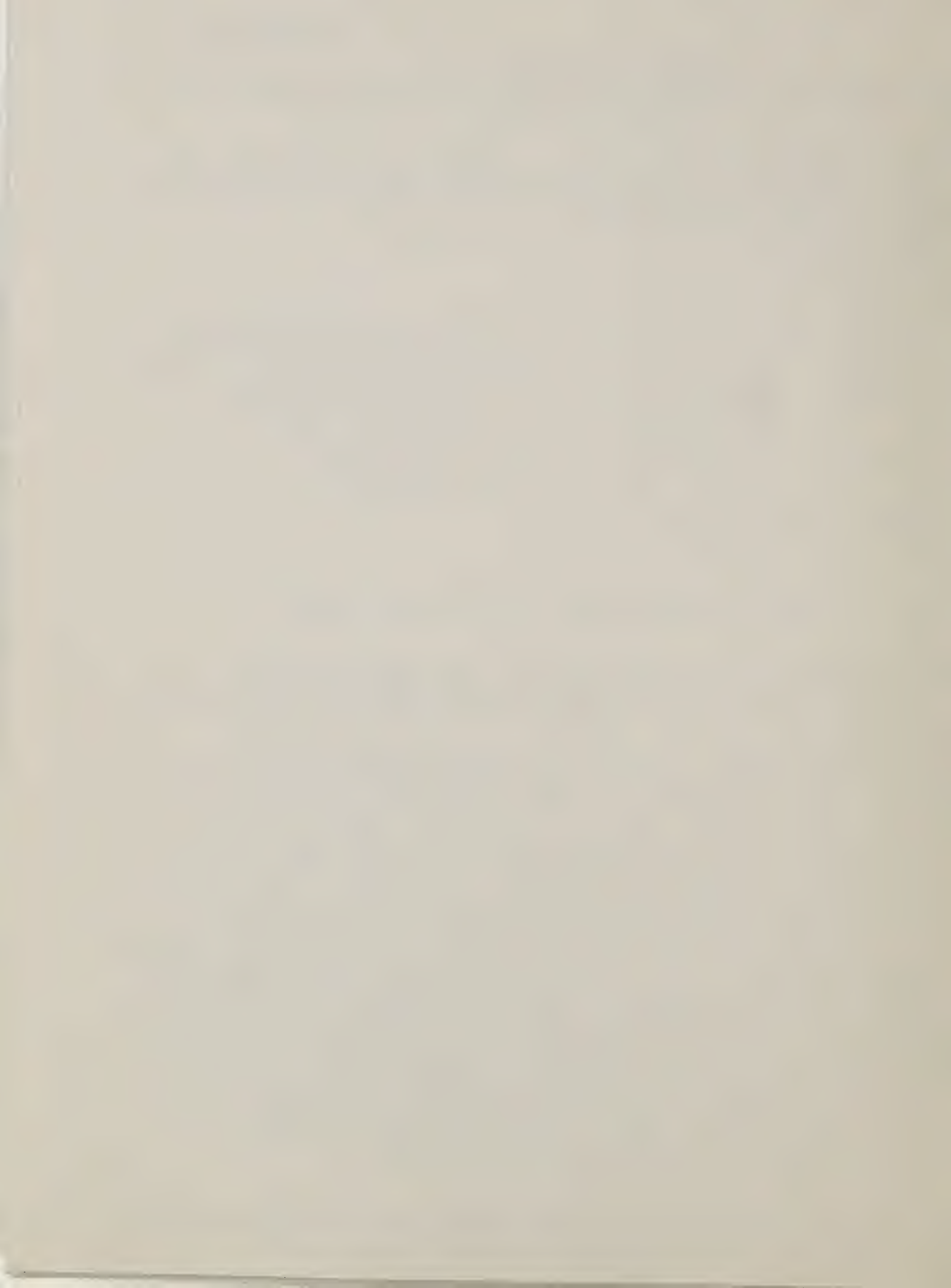
ABSTRACT AND ABSTRACT HEADING (see instruction sheet for example)

Characterization of LH-hCG receptor in testis of boars and its relationships with morphological and steroidogenic characteristics during development. J. G. Berardinelli*, J. D. Aldrich and J. J. Ford, Roman L. Hruska U.S. Meat Animal Research Center, USDA, AR, Clay Center, NE.

Objectives of this study were: 1) to characterize LH-hCG receptors of testis in boars during sexual maturation, and 2) correlate temporal changes in LH-hCG receptors with temporal changes in morphological and steroidogenic characteristics of the developing testis of boars. Testicular tissue (1g) was obtained from one testis of crossbred boars at 70, 100, 130, 160, 190, 220 and 250 days of age (5 boars/age). Binding studies using ¹²⁵I-hCG(SA = 20 to 30 uCi/ug), included maximum specific binding (MSB) and saturation analysis for estimation of number and affinity (K_a) of binding sites. MSB per gram of tissue exhibited a peak at (40±6%; P<.05) at 100 days of age. MSB per testis increased linearly (P<.05) from 190 days and plateaued by 250 days; reflective of an increase (linear) in testis weight. MSB per gram of tissue was not associated with serum testosterone (T) or in *in vitro* T production (P>.05); which occurred at 130 days. MSB per leydig cell increased linearly (P<.05) to a peak at 160 days and decreased through 250 days and was not associated with *in vitro* T production. No difference in receptor affinity was observed in these age groups and averaged 8+3x10⁹M⁻¹. Binding capacity (fM/mg ptn) was greatest at 130 days, coincident with greatest MSB per gram of tissue. Binding capacity of leydig cells was higher (P<.05) at 160 days than at other ages. The pattern of LH-hCG receptor sites per testis increased (P<.05) from 70 to 160 days and remained at this level through 250 days. LH-hCG receptors sites per leydig cell was highest at 160 days of age (P<.05) and other age groups did not differ. These differences are reflective of changes in number and volume of leydig cells, and may be related to change in serum T during development.

KEY WORDS: testis, LH-hCG receptor, boars, leydig cell, testosterone

Abstract accepted March 1, 1982, edited and ready for photocopy. Mail original and one copy to L. E. Omstedt, three different copies to program chairman of first choice and one copy to chairman of second choice, if applicable. Program chairman indicated preference for Abstract Presentation. If withdrawal of paper becomes necessary, notify L. E. Omstedt immediately.



Final Progress Report of Activities Conducted by Rodney D. Allrich While Supported by the USDA Cooperative State Research Service Grant 701-15-42 and Specific Cooperative Agreement No. 58-519B-0-899.

This report will briefly outline the series of activities and accomplishments that I was involved with while a predoctoral student at the University of Nebraska and the RLH US Meat Animal Research Center (MARC).

I started my predoctoral program in Sept., 1978 at the University of Neb. (at Lincoln). Early on, with the encouragement of Drs. Ford, Christenson and Zimmerman, one-day conferences were held between the above named persons and myself. The purpose of these bimonthly meetings was to establish guidelines for my course of study and research effort. These meetings (held alternately at Lincoln and Clay Center) were extremely beneficial. While at Lincoln, I conducted two extensive rat experiments utilizing Dr. Dickerson's rat colony.

In January 1980, after 3 semesters of course work, I moved to Clay Center to begin my period of research at MARC. While at MARC, my main research effort involved characterizing pubertal development of the boar. I also conducted several studies not directly involved with my main Ph. D. research topic. This experience was beneficial and helped broaden my overall experience. In Nov., 1981, I left MARC after obtaining a postdoctoral position at Purdue University.

During my predoctoral program, I authored or co-authored 12 abstracts and 5 manuscripts (see attachments). In addition, I attended 9 scientific society meetings and the 9th Annual Training Course for RIA sponsored by the Endocrine Society.



Overall, I believe the research environment at MARC was excellent. Adequate animal numbers, equipment and personnel were on hand to facilitate the research effort. In addition, I hereby acknowledge the generous and competent assistance of Drs. Zimmerman, Christenson and Ford during my program. Thanks also need to be given to Drs. Lunsford and Lindvall for their helpful suggestions. The able technical assistance of Mark Anderson, Toni Tolles, Ann Hultine and Jenell Dague is appreciated. I believe one of the strong points of MARC is the large number of knowledgeable personnel on staff (both scientific and technical).

When travel to Lincoln was necessary for seminars or library work, MARC generously provided transportation. Participation in seminars held within area disciplines at MARC was encouraged by the scientists and was both rewarding and helpful. By giving seminars at MARC, I improved my overall communicative skills greatly.

Students located at MARC should have sufficient self-motivation to carry on research activities without constant "prodding" by the scientist. If this was not the case, the student could easily become "lost" in the immensity of MARC. In other words, this program needs to be selective when examining prospective predoctoral students. The research topic needs to be defined and "hashed about" well before the student arrives at MARC. This will provide for efficient use of research time while at MARC.

I would like to take a moment and express my sincere thanks to Dr. Ronald Christenson for the superb manner in which he advised, encouraged and supported my efforts at MARC. His knowledge as a scientist and friend enabled me to more fully develop both professionally and personally.

I hope this summary indicates to you that:

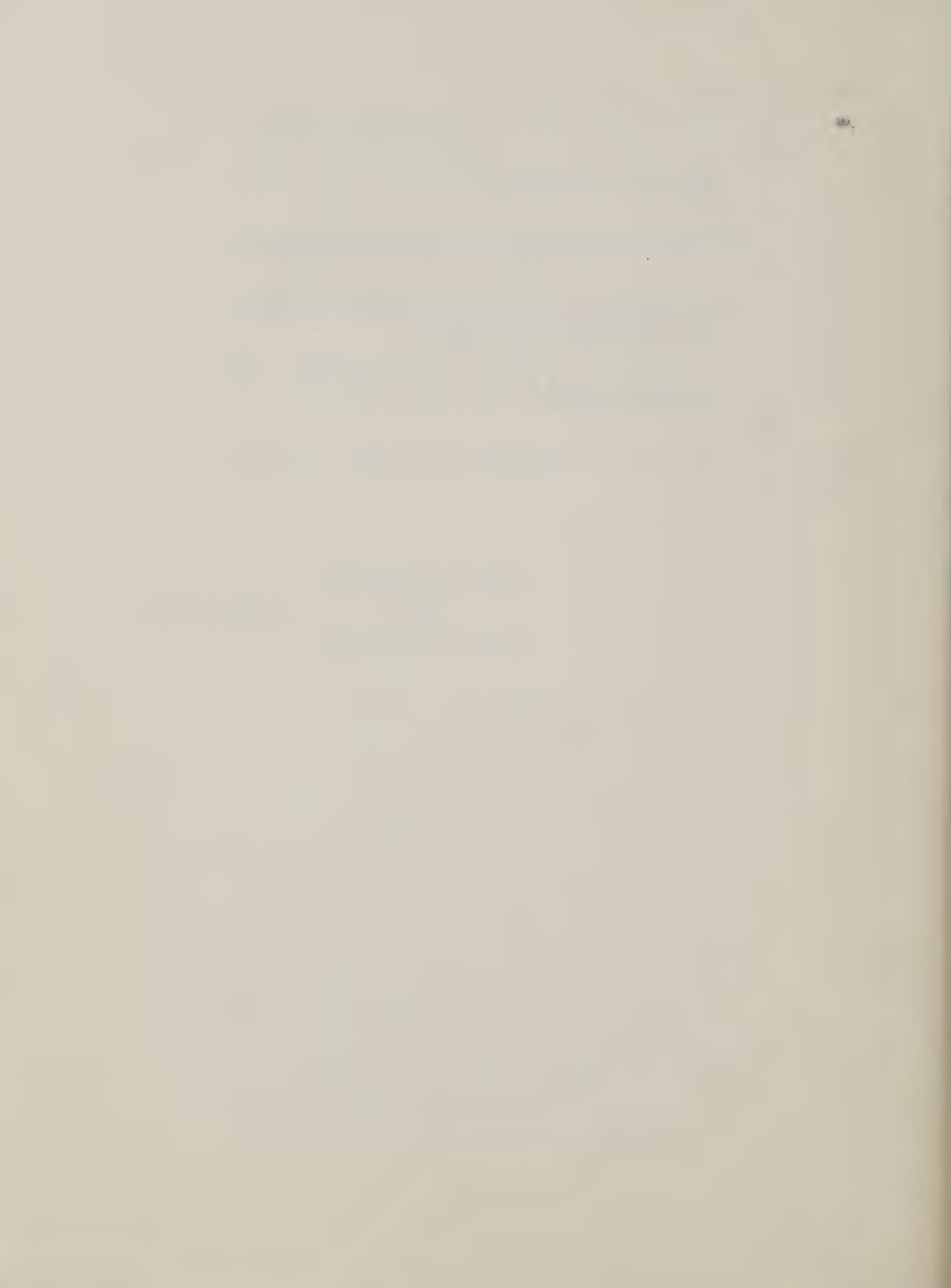
- 1) This program should be continued and expanded.
- 2) MARC is an excellent place for gaining research experience and skill.
- 3) Overall, the personnel at MARC were generous and sincere in their effort to enrich my program.
- 4) Predoctoral students need to develop a research outline and gain knowledge of previous related work before arriving at MARC.
- 5) Students must be self-motivated and capable of independent effort.

Finally, Dr. Oltjen is to be congratulated for the fine "ship" that he is running.

Sincerely Submitted,

A handwritten signature in cursive script that reads "Rodney Allrich".

Rodney D. Allrich



SELECTION FOR INCREASED RATE OR EFFICIENCY OF LEAN GROWTH IN RATS: CORRELATED RESPONSES IN REPRODUCTIVE PERFORMANCE¹

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Summary

Two lines of rats selected for either rate or efficiency of postweaning lean growth and a control line were evaluated to determine whether any differences in reproductive performance had accompanied the genetic improvement in rate or efficiency of lean growth after 14 generations of selection (followed by six generations of relaxed selection). All progeny were reared in litters of nine until weaning at 21 days of age, and in like-sex or unlike-sex pairs thereafter. Like-sex paired females were placed with unrelated males of similar age for mating at approximately 70 days of age, while unlike-sex paired females remained with the original unrelated male until mated or autopsied at 85 days of age. Selection for either rate or efficiency of lean growth increased age at vaginal opening (38.9 and 38.6 vs 36.9 days for controls; $P < .01$) and weight at vaginal opening (133.1 and 126.6 vs 119.8 g for controls; $P < .01$) in like-sex paired females; similar findings were made for unlike-sex paired females. Ovulation rate, number of fetuses and pre- and postimplantation losses were not altered by selection. Age at first spontaneous mating did not differ among lines of male rats.

Testes weight at 85 days of age were lower ($P < .01$) for males selected for efficiency of lean growth. The results indicate that there are alterations in reproductive characteristics as a consequence of selection for either rate or efficiency of lean growth in rats.

(Key Words: Rats, Selection, Growth, Correlated Responses, Reproductive Traits.)

Introduction

Selection studies at the University of Nebraska (Gosey, 1974; Notter *et al.*, 1976; Wang, 1977) and elsewhere (Krider *et al.*, 1946; Roberts, 1966a,b, 1967a,b; Robinson and Bradford, 1969; Sutherland *et al.*, 1970; Newman *et al.*, 1973; Baker *et al.*, 1975; Frahm and Brown, 1975; Bakker *et al.*, 1977) have demonstrated that rate and efficiency of growth are responsive to selection. However, few of these studies have evaluated the influence of growth selection on reproductive performance.

Fowler and Edwards (1960), Edwards (1962) and Land (1970) have examined reproductive traits in mice as affected by selection for body size. Fowler and Edwards (1960) found that fertility (defined as the interval between pairing of males and females and the arrival of the subsequent litter) was maintained in one strain of mice but was lowered in another (N). In strain N, low libido of certain males led to either total sterility or a delay in the arrival of litters. Fowler and Edwards also found that the number of ova recovered after natural mating was higher in large than in small mice. Edwards (1962) found that, in mice selected for large body size, pituitary weights and body weights were highly correlated. Land (1970) concluded that ovulation rate and both of its components (follicle stimulating hormone activ-

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TABLE 1. AGE (AVO) AND WEIGHT (WVO) AT VAGINAL OPENING AS AFFECTED BY LINE AND SEX GROUPING DURING REARING^a

| Selection line | Like-sex paired | | | Unlike-sex paired | | |
|---|-----------------|------------------------|--------------------------|-------------------|------------------------|--------------------------|
| | No. of females | AVO, days | WVO, g | No. of females | AVO, days | WVO, g |
| LG (selected for rate of lean growth) | 103 | 38.9 ± .4 ^b | 133.1 ± 2.0 ^b | 21 | 39.7 ± .8 ^c | 135.6 ± 3.4 ^c |
| LE (selected for efficiency of lean growth) | 115 | 38.6 ± .4 ^b | 126.6 ± 1.6 ^b | 21 | 38.3 ± .7 | 133.3 ± 4.7 ^c |
| C (control) | 113 | 36.9 ± .4 | 119.8 ± 1.4 | 28 | 37.0 ± .7 | 120.1 ± 2.9 |

^aData presented are least-squares means ± SE.

^bDifferent ($P < .01$) from C value.

^cDifferent ($P < .05$) from C value.

ity and ovarian sensitivity) are positively correlated with body weight.

The objective of this study was to evaluate the change in rat reproductive traits that had occurred after long-term selection for either rate or efficiency of lean growth.

Materials and Methods

Rats that had undergone 14 generations of selection for either rate (LG) or efficiency (LE) of postweaning (3 to 8 weeks of age or constant final weight) lean growth followed by six generations of relaxed selection (generations 15 to 21) were utilized. A randomly selected control line (C) was also maintained in each generation. Lines LG and LE were duplicated in each generation, whereas the C line was triplicated. The selection procedures and growth responses have been reported elsewhere (Gosey, 1974; Notter *et al.*, 1976; Wang, 1977, 1981). Briefly, selection has resulted in increased rate (5.41, 5.10 and 4.19 g/day) and efficiency (.328, .325 and .286 g/g feed) of lean growth in the selected lines (LG, LE and C, respectively). The genetic gain achieved during selection has been essentially maintained during relaxed selection. Selection has not resulted in any significant trend in litter size changes.

Standardized litters of nine rats (five females and four males) were maintained from 6 days of age whenever possible. The laboratory was maintained at 26 C and 45% relative humidity,

with 12 hr of light/day (0630 to 1830 hr). A commercial⁵ cubed diet containing 24% crude protein, 4% crude fat and 4.5% crude fiber was provided *ad libitum*.

After weaning at 21 days of age, female rats were housed either in like-sex pairs with a sibling female or in unlike-sex pairs with an unrelated male of similar age and line. Male weanling rats were housed either in like-sex pairs with an unrelated male or in unlike-sex pairs with an unrelated female of similar age and line. Sex groupings were maintained until mating or autopsy.

Starting at 28 days of age, all females were examined for vaginal opening (VO) every other day, with age (AVO) and weight (WVO) recorded when VO occurred. VO was used as a measure of the onset of puberty. It had been verified for these lines of rats that vaginal estrus occurred within 1 day of VO and that the first ovulation accompanied the first estrous smear.

The number of females on test was reduced after VO (randomly within line) because the animals were needed for other experiments. Mating was initiated at 70 days of age by caging like-sex paired females individually with like-sex paired males. Across-line mating was not allowed. A sperm-positive vaginal smear was taken as an indication that mating had occurred. Unlike-sex paired females remained with their original male companion until a sperm positive smear was observed or until termination of the experiment. The presence of spermatozoa in the smear denoted the first spontaneous mating for the male and day 0 of pregnancy for the

⁵ Wayne Lab-blox, Allied Mills, Inc., Chicago, IL.

TABLE 2. OVULATION RATE (OR), NUMBER OF FETUSES (NF) AND PRE- AND POSTIMPLANTATION LOSSES IN LIKE-SEX PAIRED FEMALE RATS^a

| Selection line | No. of females | OR | NF ^b | Preimplantation loss ^b | Postimplantation loss ^b |
|---|----------------|-----------|---------------------|-----------------------------------|------------------------------------|
| LG (selected for rate of lean growth) | 42 | 15.3 ± .5 | 13.3 ± .4 (86.9) | 1.2 ± .3 (7.8) | .8 ± .2 (5.3) |
| LE (selected for efficiency of lean growth) | 40 | 14.1 ± .3 | 12.6 ± .4 (89.4) | 1.0 ± .2 (7.1) | .5 ± .1 (3.5) |
| C (control) | 39 | 14.7 ± .4 | 12.9 ± .5 (87.8) | 1.1 ± .3 (7.4) | .7 ± .2 (4.8) |

^aData presented are least-squares means ± SE.^bNumber in parenthesis represents percentage of OR.

female. The age and weight of the male were taken at this time. All males were sacrificed at 85 days of age, with body weight (BW) and testes weight (TW) recorded.

Both sets of females were sacrificed at day 16 to 18 of gestation, and number of corpora lutea (ovulation rate, OR), number of fetuses (NF) and number of resorptions (NR) were counted and recorded. Preimplantation loss was calculated as the difference between OR and the sum of NF and NR. Preimplantation loss included the loss due to fertilization failure and early embryonic mortality. Postimplantation loss represented the difference between number of implantation sites and number of normal 18-day fetuses.

The data were analyzed by least-squares

fixed-model procedures (General Linear Models procedure, option 1; Barr *et al.*, 1979). The model for analyses of all traits included fixed effects of line, sex grouping and the interaction of line and sex grouping. In addition, data from the like-sex paired female group were analyzed with line and sibling within line used as fixed effects. The residual mean square was used as the error term to test the significance of main effects, the interaction term and appropriate linear contrasts. The sibling-within-line mean square was used as the error term in the analysis of like-sex paired female traits. Variation in *n* values between data tables was the result of death losses, mating failures and failure to detect specific monitored events in some animals (i.e., first spontaneous mating). Since

TABLE 3. OVULATION RATE (OR), NUMBER OF FETUSES (NF) AND PRE- AND POSTIMPLANTATION LOSSES IN UNLIKE-SEX PAIRED FEMALE RATS^a

| Selection line | No. of females | OR | NF ^b | Preimplantation loss ^b | Postimplantation loss ^b |
|---|----------------|-----------|---------------------|-----------------------------------|------------------------------------|
| LG (selected for rate of lean growth) | 18 | 15.9 ± .8 | 12.3 ± .9 (77.4) | 2.7 ± .7 (17.0) | .9 ± .2 (5.6) |
| LE (selected for efficiency of lean growth) | 19 | 15.5 ± .6 | 11.4 ± .8 (73.6) | 3.1 ± .6 (20.0) | 1.0 ± .4 (6.4) |
| C (control) | 27 | 16.4 ± .7 | 13.0 ± .8 (79.3) | 2.8 ± .5 (17.1) | .6 ± .2 (3.6) |

^aData presented are least-squares means ± SE.^bNumber in parenthesis represents percentage of OR.

TABLE 4. AGE AND WEIGHT AT FIRST SPONTANEOUS MATING AS AFFECTED BY LINE IN UNLIKE-SEX PAIRED MALE RATS^a

| Selection line | No. of males | Age at first spontaneous mating, days | Weight at first spontaneous mating, g |
|---|--------------|---------------------------------------|---------------------------------------|
| LG (selected for rate of lean growth) | 13 | 62.3 ± 1.6 | 339 ± 10 |
| LE (selected for efficiency of lean growth) | 19 | 61.5 ± 1.4 | 306 ± 10 ^b |
| C (control) | 23 | 61.9 ± 1.1 | 294 ± 11 ^b |

^aData presented are least-squares means ± SE.

^bDifferent ($P < .05$) from LG value.

no differences were present in the duplicate (LG, LE) or triplicate (C) lines within the respective selection lines, the data from any given selection line were analyzed without regard to duplicate or triplicate line designation.

Results

The responses of the female rats, in terms of AVO and WVO, to selection and sex grouping during development are summarized in table 1. Selection for either LG or LE delayed ($P < .01$) AVO and increased ($P < .01$) WVO in like-sex paired female rats. The results were similar for unlike-sex paired females. Comparison of the two sexual groupings of females showed no significant differences in these traits. Also, no significant interactions were found between line and sex grouping for either AVO or WVO. Overall, there was a significant positive correlation ($r = .53$, $P < .001$) between AVO and WVO. Of 402 females, only one did not exhibit VO.

Selection for either LG or LE did not alter OR as compared with OR in the randomly selected line C (tables 2 and 3). OR was higher ($P < .05$) among unlike-sex paired females than among females reared in a like-sex pair.

NF at 18 days of gestation did not differ among lines in either of the sex groupings (tables 2 and 3). Among the like-sex paired females, the largest difference (.7 fetuses) was observed between the LG and LE lines. Among the unlike-sex paired females, the largest difference (1.6 fetuses) was between the LE and C lines. NF was not significantly affected by sex grouping or by the interaction between sex grouping and line.

Neither pre- nor postimplantation loss dif-

fered between lines. Preimplantation loss was two to three times higher ($P < .05$) for the unlike-sex paired females than for the like-sex paired group. The difference averaged 1.7 embryos overall. Postimplantation loss was similar for like- and unlike-sex paired females. Conception rates did not differ between lines or sex groupings (93, 94 and 96% for LG, LE and C, respectively).

Age of males at first spontaneous mating was similar for all lines, averaging approximately 62 days (table 4). However, LG males were heavier ($P < .05$) in BW at this time than either LE or C male rats.

Selection for efficiency of lean growth (LE) results in smaller testes ($P < .01$) at 85 days of age than in lines LG and C (table 5). When TW was adjusted by a covariable analysis for BW, results still showed that the LE males had smaller ($P < .01$) testes. BW was higher ($P < .01$) in the LG line than in either the LE or the C line. Sex grouping did not influence TW.

Discussion

The data presented in this paper demonstrate that selection for either rate or efficiency of lean growth may alter specific reproductive traits of both female and male rats. Other researchers have also reported alterations in reproductive characteristics as a result of selection for growth rate. Therefore, in selection studies involving growth rate, it is critical that reproductive traits be monitored so that beneficial and/or detrimental correlated responses be identified.

The results show that AVO was delayed and WVO increased in lines LG and LE as compared

TABLE 9. TESTES WEIGHT (TW) AND BODY WEIGHT (BW) AT 85 DAYS OF AGE AS AFFECTED BY LINE AND SEX GROUPING DURING REARING IN MALE RATS^a

| Selection line | Like-sex paired | | | Unlike-sex paired | | |
|--|-----------------|-------------------------|----------------------|-------------------|-------------------------|-----------------------|
| | No. of males | TW, g | BW, g | No. of males | TW, g | BW, b |
| I.G (selected for rate of lean growth) | 32 | 3.69 ± .07 ^b | 420 ± 8 | 20 | 3.64 ± .09 ^c | 436 ± 12 |
| I.E (selected for efficiency of lean growth) | 42 | 3.31 ± .05 | 389 ± 7 ^d | 20 | 3.39 ± .08 | 393 ± 8 ^d |
| C (control) | 43 | 3.54 ± .07 ^b | 380 ± 8 ^d | 22 | 3.54 ± .07 ^c | 382 ± 10 ^d |

^aData presented are least-squares means ± SE.

^bDifferent (P<.01) from LE value.

^cDifferent (P<.05) from LE value.

^dDifferent (P<.01) from LG value.

with line C. Selection for LG or LE slowed the sexual maturation process in some manner. Since the LE line also had an increase in growth rate, the delayed AVO for both LG and LE lines may be related to increases in growth rate. There are conflicting views about the relationship between BW and the occurrence of puberty. Norris and Adams (1979) have stated that accelerated growth in the rat is not correlated with accelerated sexual maturation. The present data are in agreement with that statement. It may be that puberty is attained when the body mass reaches a critical percentage relative to the mature body mass characteristic of the given animal. Further studies are needed to delineate any such relationships.

The presence of the male did not accelerate or delay AVO or alter WVO in the present experiment. This observation is in agreement with that of Norris and Adams (1979), who found that keeping a mature male with a weanling female rat advanced neither age at VO nor age at first estrus. In mice, sexual maturation of females has been expedited by the presence of an adult male mouse and by bedding soiled by an adult male (Vandenbergh, 1967, 1969).

Selection for either LG or LE did not alter OR, NF or pre- and postimplantation loss. Fowler and Edwards (1960), Land (1970) and Bradford (1971) have reported that OR increases in response to selection of postweaning gain in mice. Land (1970) concluded that in the mouse there is a positive genetic relationship

between BW and OR and between BW weight and ovarian sensitivity (as measured by the response to PMS). Bradford (1971) also found pre- and postimplantation losses to be higher in lines selected for rapid postweaning gain. Litter size remained unchanged, apparently because "uterine capacity" was not sufficient to accommodate the additional embryos. The failure of OR to increase in LG and LE lines differs with findings from the cited mouse studies. Further studies are needed with rats of varying genetic composition to determine whether the responses of mice and rats differ in this respect.

Rearing female rats with a male from weaning increased (P<.05) OR in the present study. The females reared with males were mated at a younger age (62 days) than like-sex females (72 days), or the difference might have been greater. This finding was surprising in view of the failure of the male's presence to expedite the onset of puberty in the same females. Although the male has been shown to stimulate earlier puberty in female mice (Vandenbergh, 1967), the influence of the male on OR has not been thoroughly evaluated.

Females reared with males produced (P<.01) smaller litters than like-sex paired females as the result of greater preimplantation loss. The greater loss may have been due to two factors. First, the unlike-sex paired females were younger when mated and might not have possessed the uterine capacity of older females. Secondly, these females were mated to younger males that probably had not attained a maximal

level of fertility. The design of this experiment did not allow identification of the specific reproductive deficiency responsible for this difference.

Male reproductive responses to growth selection have not been examined to the same degree as female responses. Usually, all that is stated is that the males were able to mate females and fertility was normal. In this study, males were evaluated for age at first spontaneous mating and TW at 85 days of age.

Age at first spontaneous mating was thought to reflect the onset of the male's ability to mate, since the females that were housed with males (unlike-sex paired) exhibited VO (38 days) long before mating occurred. As the males were of similar age, the lack of mating activity after the females experienced VO should have been indicative of the male's nonbreeding activity. Therefore, when mating did occur, it was assumed to signal the onset of the male's mating ability. Age at first spontaneous mating was similar for all lines, indicating that this variable is not influenced by selection for rate or efficiency of lean growth. Weight at first spontaneous mating was increased in the LG line, as expected, because LG rats had the highest BW of the three lines at any given age. This suggests that age of the male may be a more important factor than BW in determining the onset of mating ability.

Selection for efficiency of lean growth (LE) significantly reduced TW, but this decrease apparently did not influence the male's ability to impregnate females. However, under the experimental procedures, males were only required to mate one female and were not put through an exhaustive mating scheme. The TW response of the LE rats was independent of direct BW influence and therefore leads one to believe that some endocrine mechanism was a dominant factor in this effect. TW of LG line males was not altered by selection. Johnson and Eisen (1975) have reported increased TW in mice selected for postweaning gain; however, when expressing TW per gram BW, they actually found TW decreased per unit BW. In the present study, this effect was not detected. It is of interest to note that both TW and OR were lowest in the LE line. The decreased TW of the males and the decreased OR of the females in this selected line (LE) may have been related to the amount of gonadotropin released.

This study has shown that selection for LG or LE altered AVO and WVO of the female and

that LE selection reduced TW of the male. Rearing female rats with a male from weaning to mating resulted in an increased OR independent of BW changes.

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PUBERTAL DEVELOPMENT OF THE BOAR: TESTOSTERONE, ESTRADIOL-17 β , CORTISOL
AND LH CONCENTRATIONS BEFORE AND AFTER CASTRATION
AT VARIOUS AGES^{1,2,3}

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Summary

Forty-eight Landrace X Duroc boars were assigned at weaning to eight castration ages (40, 70, 100, 130, 160, 190, 220 and 250 d). Catheterization of the external jugular vein was performed 5 d before scheduled castration. Blood samples were collected every .5 h between 0800 and 1200 h 2 d before (-2) and on d +1, +2, +3, +4, +8 and +16 after castration. Testosterone (T) was determined in serum samples collected every .5 h on d -2. Serum concentrations of estradiol-17 β (E₂), cortisol (C) and luteinizing hormone (LH) were quantified by radioimmunoassays in pooled (within boar) samples collected on d -2. In addition, LH was determined in pooled (within boar) samples collected on d +1, +2, +3, +4, +8 and +16 after castration. Mean concentrations of T and E₂ (d -2) increased in a near linear ($P < .01$) fashion with age of boar. However, T concentrations at 250 d of age had declined ($P < .01$) to values similar to concentrations of boars at 130 d of age. Serum C concentrations (d -2) were lower ($P < .01$) at 100, 130 and 160 d of age when compared with an average C concentration of 19 ng/ml for all other age groups. Mean concentrations of LH (d -2) did not differ among any of the ages. Luteinizing hormone concentrations were elevated ($P < .01$) within 1 to 2 d following castration at 40, 70, 100, 130 and 160 d of age. Serum T concentrations of individual boars varied greatly at all ages as determined by half-hourly sampling over a 4-h period. The results indicate that in the boar: (1) T concentrations increase as pubertal development progresses and decline as maturity nears; (2) E₂ concentrations increase steadily through pubertal development; (3) C concentrations are depressed at 100, 130 and 160 d of age relative to concentrations before and after that period; (4) LH concentrations were constant during pubertal development, and (5) a decrease in

the sensitivity of the negative feedback mechanism controlling LH occurs after 160 d of age.

(Key Words: Boar, Puberty, Testosterone, Estradiol-17 β , Cortisol, Luteinizing hormone)

Introduction

Few studies have comprehensively examined patterns of hormone secretion during pubertal development of the boar. Studies (Colenbrander et al., 1978; FlorCruz and Lapwood, 1978; Lapwood and FlorCruz, 1973; Tan and Raeside, 1980) have not yielded consistent evidence as to the patterns of testosterone (T) secretion during development. In general, T concentrations appear to increase in sera until about 170 to 230 d of age and then decline to concentrations found in the adult. Plasma estradiol-17 β (E₂) concentrations have been reported (Claus and Hoffmann, 1980; Hay et al., 1981) to be as high as 278 pg/ml in adult boars. Plasma concentrations of cortisol (C) in mature boars range between 10 and 25 ng/ml (Liptrap and Raeside, 1975, 1978).

Although patterns of Luteinizing hormone (LH) secretion during development have been studied (Romanowicz et al., 1976; FlorCruz and Lapwood, 1978) in boars, due to variation in results, it could not be ascertained if LH concentrations were altered during pubertal development. Luteinizing hormone concentrations are thought to be controlled through a negative feedback system involving testicular steroids. Castration of pubertal boars results in elevated levels of LH within 2 to 4 wk (Colenbrander et al., 1977; FlorCruz and Lapwood, 1978), but characterization of the LH response to castration during pubertal development is incomplete. Serum T concentrations in boars are characterized by episodic fluctuations that seem more pronounced before than after puberty (Brock and Wettemann, 1976; Sanford et al., 1976; Lapwood and FlorCruz, 1978; Kattesh et al., 1979; Tan and Raeside, 1980).

The objective of the present investigation was to characterize patterns of serum T, E₂, C and LH concentrations during pubertal development and to evaluate negative feedback regulation of LH by determining serum concentra-

tions before and after castration of the boar. In addition, episodic fluctuations in T concentrations were examined over a 4-h period before castration for all boars.

Materials and Methods

Forty-eight Landrace X Duroc boars were reared in standard management conditions in a total confinement environment. Boars were born within a 3-wk period, weaned at 4 wk of age and maintained in a nursery in homosexual groups until 10 wk of age. Boars were then moved to a modified-open-front (MOF) type of finishing building and penned in groups of 16. At about 150 d of age, boars were moved to individual stalls. Diets with a corn and soybean meal base containing 18.5% protein were fed to boars in the nursery, whereas boars in MOF and stalls were fed diets with 16% protein. Ad libitum feeding was practiced until boars were moved to individual stalls where 2.8 kg feed was available daily. Boars were exposed to 12 h of light daily in the farrowing house and nursery and 16 h of light daily through the remainder of the experiment.

At weaning, boars were randomly allotted in a randomized block design (six sire groups) to eight castration ages (40, 70, 100, 130, 160, 190, 220 and 250 d). Five days before scheduled castration, catheterization of the external jugular vein (Ford and Maurer, 1978) was performed for the collection of blood samples. At castration, anesthesia was induced and maintained with sodium thiopental iv. Testes and epididymides were promptly removed, trimmed of excess tissue and weighed separately. Blood samples were taken every .5 h between 0800 and 1200 h 2 d before (-2) and on d +1, +2, +3, +4, +8 and +16 after castration. Blood samples were allowed to clot for at least 24 h at 4 C, centrifuged at 2,000 X g for 30 min and the serum fraction

pipetted and stored at -20 C until all samples could be assayed simultaneously for the same hormone. At the time of serum removal, a pooled composite sample was made from the .5-h samples collected each day from individual boars. Testosterone was determined on every .5-h sample collected on d -2. Concentrations of E₂ and C were determined on pooled d -2 samples and concentrations of LH were determined on all pooled samples from all days.

Concentrations of T were determined in duplicate 150- μ l aliquots of serum by radioimmunoassay (RIA) as described by Ford et al. (1980) using a rabbit antiserum⁸ prepared against testosterone-19-bovine serum albumin. This antiserum had less than 10% crossreactivity with 5 α -dihydrotestosterone. Samples were extracted twice by mixing with ethyl ether for 15 min, freezing and then decanting the ethyl ether. Recovery averaged 92 to 95% and samples were not corrected for procedural losses. Standard curves of T⁹ ranged from 10 to 4,000 pg. Intra- and interassay coefficients of variation were 7.5 and 10.5%, respectively.

Concentrations of E₂ were determined in duplicate 500- μ l aliquots of serum by RIA as described by Kesler et al. (1977) using an antiserum¹⁰ developed in rabbits prepared against estradiol-17 β -6-bovine serum albumin. The RIA was validated for swine by Redmer and Day (1981). Samples were extracted twice by mixing with ethyl ether for 15 min, freezing and then decanting the ethyl ether. Recovery averaged 93% and samples were not corrected for procedural losses. Standard curves of E₂⁹ ranged from .78 to 200 pg. Intra- and interassay coefficients of variation were 8.1 and 9.5%, respectively.

⁸ Cambridge Nuclear Radiopharmaceutical Corp., Billerica, MA, cat. #CNR-248

⁹ Sigma Co., St. Louis, MO

¹⁰ Kindly provided by Dr. Norman Mason, Eli Lilly Co., Indianapolis, IN.

Concentrations of C were determined in duplicate 10- μ l aliquots of serum by RIA (Dash et al., 1975) using a rabbit antiserum¹¹ prepared against cortisol-21-human serum albumin. This antiserum had 11 and 58% crossreactivity with corticosterone and 11-deoxycortisol, respectively. Samples were extracted twice by mixing with methylene chloride for 15 min, freezing and then decanting the methylene chloride. Recovery averaged 92% and samples were not corrected for procedural losses. Standard curves of C⁹ ranged from 25 to 2,000 pg. Intra- and interassay coefficients of variation were 6.9 and 9.6%, respectively.

Concentrations of LH were determined in duplicate 300- μ l aliquots of serum by a double-antibody RIA described by Niswender et al. (1969) using anti-porcine LH (#556) as first antibody and sheep anti-rabbit gamma globulin as second antibody. Purified porcine LH (LER-786-3) labeled with ¹²⁵I by the chloramine T method (Greenwood et al., 1963) and separated on a Bio-Gel P-100 column served as trace. Samples were assayed against standard curves prepared from LER-786-3, that ranged from .0625 to 20.0 ng. Intra- and interassay coefficients of variation were 8.4 and 9.3%, respectively.

The data were analyzed by least-squares fixed-model procedures (General Linear Models procedure; Barr et al., 1979). The model for analysis of all traits, except LH responses to castration, included fixed effects of age and sire. The model for analysis of LH responses to castration involved a split-plot analysis with age as the main plot treatment and day relative to castration as the subplot treatment. Differences between treatment means were determined by the Newman-Keuls test (Steel and Torrie, 1960).

¹¹ Cambridge Nuclear Radiopharmaceutical Corp., Billerica, MA, cat. #CNR-207

Results

Mean body weights and paired weights of testes and epididymides increased throughout the period of sampling from 40 to 250 d of age (figure 1). Relative growth rate, which represents the percentage change in body or organ weight/day (Fitzhugh and Taylor, 1971), steadily decreased for body and epididymides weight during pubertal development, whereas relative growth rate for testes increased markedly between 100 and 130 d of age and then steadily decreased thereafter (table 1). Within each age group, Pearson correlations ($r = .50^{\circ}$, $P > .30$) indicated that neither testes nor epididymides weight was correlated with body weight. Analysis of variance indicated that sire influenced ($P < .01$) both testes and epididymides weight.

Average serum T concentrations ranged between 1.31 and 15.76 ng/ml from 40 to 250 d of age (table 2). Serum T increased linearly ($P < .01$) with age of boar and was not influenced by sire. Serum T concentrations at 250 d of age were similar to those found at 130 d of age. Serum T concentrations of samples collected every .5 h on d -2 displayed episodic fluctuations for all ages examined. Mean number of T peaks/4 h ranged between 1.0 and 1.7 for all ages and no clear age-trends in fluctuations were detected. Within age group, wide variations in T fluctuations were noted, with some boars displaying distinct spikes in secretory profiles while others did not.

Mean serum E_2 concentrations increased in a near linear fashion ($P < .01$) with age from 11.3 pg/ml at 40 d of age to 114.2 pg/ml at 250 d of age (figure 3). Analysis of variance indicated that E_2 concentrations were influenced ($P < .01$) by sire.

Average serum C concentrations ranged between 9.0 and 23.3 ng/ml (figure 4). Serum C was lower ($P < .01$) at 100, 130 and 160 d of age than C concen-

trations before or after this time. Sire had no effect on C concentrations.

Before castration, mean serum LH concentrations ranged between .83 and 1.25 ng/ml (figure 5). Luteinizing hormone was not influenced by sire or age of boar. Luteinizing hormone concentrations were elevated ($P < .01$) within 1 to 2 d after castration in boars 40 through 160 d of age with no such response noted in older boars (table 3). The LH concentrations after d +2 tended to decline in most cases to values near those found before castration and by d +16, only one age group (40 d) displayed elevated ($P < .01$) LH concentrations relative to d -2.

Discussion

Testicular growth displayed a sigmoid curve function similar to previous reports (Niwa and Mizuho, 1954; McFee and Eblen, 1967; Egbunike and Steinbach, 1972; FlorCruz and Lapwood, 1978), although in the present experiment, a definite plateau had not been reached by 250 d of age. Relative growth rate of the testes and the lack of significant correlations between body and testes weights within age groups indicated that growth of the testes was not directly associated with bodyweight. Sire influenced both testes and epididymides weight, suggesting that significant additive gene variation exists for these traits. In future investigations, greater sire and progeny numbers are needed to evaluate physiological mechanisms, heritabilities, genetic and phenotypic correlations among these parameters.

Testosterone concentrations increased between 40 and 220 d of age before declining sharply at 250 d of age. This pattern of T secretion is similar to those noted previously (Andresen, 1976; Romanowicz et al., 1976; Colenbrander et al., 1978; FlorCruz and Lapwood, 1978). Due to the age at termination of previous experiments, the drop we observed at 250 d has not always been

detected. The mechanism responsible for the increasing concentrations of T during pubertal development and the decline in T concentrations at 250 d can only be theorized. As the boar develops, testes weight increases, thereby providing for the presence of a larger mass of steroidogenic tissue. The authors have noted (Allrich and Christenson, 1981) that sensitivity of Leydig cells to gonadotropins may increase during pubertal development. The increased mass and sensitivity of Leydig cells may in turn produce greater quantities of steroids, resulting in higher serum concentrations of T. Metabolic clearance rate of T may also be lower at this time. Then by 250 d, after maximum growth has been obtained by seminiferous tubules and accessory sex glands, T may no longer be required in high concentrations with a new set-point being established, thus lowering T concentrations. This may be brought about by a shift in the preferred metabolic pathways involved in androgen synthesis.

The profiles of E₂ concentrations remained constant through 100 d of age and increased steadily thereafter. Serum E₂ concentrations did not decline at 250 d as did T concentrations. One possible explanation for sustained levels of E₂ is that a shift in metabolism occurs in the testes, thereby allowing E₂ secretion to continue in the face of declining T concentrations. The function of E₂ in male reproductive physiology is not well known, but a role in sexual behavior (Baum and Vreeburg, 1973; Larsson et al., 1973) and testicular T synthesis (Tcholakian et al., 1974; Moger, 1976; Dorrington et al., 1978; Jones et al., 1978) has been suggested. In the boar, Joshi and Raeside (1973) concluded that E₂ acts synergistically with T on accessory sex glands and sexual behavior. High plasma E₂ and urine estrogen concentrations of adult boars have been reported (Velle, 1958; Raeside, 1965; Claus and

Hoffman, 1980; Hay et al., 1981). In the present study, serum concentrations of E₂ were influenced by sire, indicating that sire may alter hormonal patterns in endocrine studies.

Cortisol displayed a distinct drop in concentrations (to 10 ng/ml) at 100, 130 and 160 d of age. Cortisol is the major glucocorticoid found in the blood of boars (Bottoms et al., 1972) and is secreted in response to stress-inducing situations. Serum C was monitored in the present experiment because increases in adrenal steroid secretion are associated with increases in testicular steroid secretion in boars (Liptrap and Raeside, 1975, 1978). No such association was evident in the present study.

A slight, but not significant, elevation in LH concentrations occurred at about the time of greatest testicular growth. FlorCruz and Lapwood (1978) reported that a significant elevation of LH concentrations occurred during puberty in the boar. Others (Romanowicz et al., 1976) have not observed an elevation in LH concentrations during this period. Serum LH during pubertal development may be partially responsible for growth of the testes and increasing concentrations of T. Research conducted with bulls (Lacroix et al., 1977; Lacroix and Pelletier, 1979; McCarthy et al., 1979), rams (Courot et al., 1975) and male rats (Smith et al., 1977; Grizard et al., 1980; Slob et al., 1980) have shown minor elevations in LH levels during pubertal development.

The existence of an operative negative feedback mechanism was assessed by evaluating LH concentrations before and after castration at several ages. Through 160 d of age, a negative feedback mechanism was operating as evidenced by elevated LH concentrations 1 to 2 d after castration, when compared with concentrations found before castration. This response was absent after

160 d and suggests a decrease in the sensitivity of the negative feedback mechanism. Luteinizing hormone concentrations tended to decline after d +2 and were similar to concentrations found on d -2. By d +16, LH concentrations in only the 40-d age group had increased to the point of being elevated relative to d -2. This biphasic response in LH concentrations, except for the 40-d group, has not been observed in previous studies (Colenbrander et al., 1977; FlorCruz and Lapwood, 1978) because LH concentrations were not monitored shortly after castration. In studies that examined LH concentrations shortly after castration (Ford and Schanbacher, 1977; Elsaesser et al., 1978), prolonged surveillance of LH concentrations was not conducted. The absence of elevated LH concentrations on d +16 may be explained by the fact that more time may be required to elicit sustained serum LH concentrations. In one study (Colenbrander et al., 1977), LH concentrations were slightly elevated by 14 d after castration, with further elevations observed by 28 d in boars that were 56 d of age at castration.

Boars, at all ages monitored, displayed episodic fluctuations in T concentrations. Within age group, wide variations between boars were noted. Some boars displayed very pronounced episodes of T release and subsequent decline while other boars, at the same age, showed little fluctuation over the 4-h period that was monitored. Other studies (Brock and Wettemann, 1976; Sanford et al., 1976; Claus and Gimenez, 1977; Lapwood and FlorCruz, 1978; Kattesh et al., 1979) have also observed episodic fluctuations in blood T concentrations of boars.

In conclusion, the results of this study demonstrate that marked alterations occur in serum T, E₂ and C concentrations during pubertal development of the boar. In addition, serum LH concentrations do not increase during

pubertal development, but are elevated within 2 d after castration at 40, 70, 100, 130 and 160 d of age. Also, sire was shown to influence testes and epididymides weight and serum E₂ concentrations.

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Table 1. RELATIVE GROWTH RATES OF THE BODY, TESTES AND EPIDIDYIMIDES OF BOARS DURING PUBERTAL DEVELOPMENT

| Age, d | No. ^a | Relative growth rate (% wt/d) | | |
|-----------|------------------|-------------------------------|---------------------|---------------------------|
| | | Body | Testes ^b | Epididymides ^b |
| 70 | 40 | 5.29 ± .20 | 5.57 | 6.79 |
| 100 | 34 | 2.80 ± .13 | 4.14 | 4.62 |
| 130 | 28 | 1.98 ± .05 | 10.44 | 3.56 |
| 160 | 22 | 1.20 ± .01 | 3.36 | 2.92 |
| 190 | 17 | .70 ± .08 | 1.28 | 1.82 |
| 220 | 10 | .25 ± .06 | .29 | .58 |
| 250 | 6 | .17 ± .07 | .14 | .10 |

^a For body growth only.

^b No error term possible due to single individual measurements.

Table 2. SUMMARY OF SERUM TESTOSTERONE CONCENTRATIONS AND FLUCTUATIONS IN BOARS BETWEEN 0800 AND 1200 H DURING PUBERTAL DEVELOPMENT

| Age, d | No. | Testosterone (ng/ml) | | | Mean number of peaks ^d /4 h ^a |
|-----------|-----|---------------------------|-----------------------|-----------------|--|
| | | Overall mean ^a | Baseline ^b | SD ^c | |
| 40 | 4 | 1.31 ± .16 | .76 | .84 | 1.5 ± .3 |
| 70 | 6 | 2.24 ± .17 | 1.37 | .93 | 1.7 ± .2 |
| 100 | 5 | 4.03 ± .28 | 2.28 | 1.81 | 1.0 ± 0 |
| 130 | 6 | 9.99 ± .41 | 7.88 | 2.18 | 1.7 ± .2 |
| 160 | 6 | 10.92 ± .30 | 8.96 | 2.14 | 1.0 ± .3 |
| 190 | 6 | 15.75 ± .77 | 10.99 | 4.34 | 1.5 ± .3 |
| 220 | 6 | 15.76 ± .79 | 11.78 | 4.31 | 1.5 ± .2 |
| 250 | 5 | 8.66 ± .63 | 6.61 | 2.44 | 1.3 ± .3 |

a Least-squares means ± SE.

b Baseline defined as mean of three lowest samples collected during 4 h.

c Standard deviation of overall mean.

d Peak defined as value greater than 2 SD above baseline.

Table 3. EFFECT OF CASTRATION ON SERUM LH CONCENTRATIONS OF BOARS BETWEEN 40 AND 250 D OF AGE.

| Age, d | Days from castration | | | | | | |
|-----------|----------------------|--------------|--------------|--------------|------------|-------------|--------------|
| | -2 | +1 | +2 | +3 | +4 | +8 | +16 |
| | ng/ml | | | | | | |
| 40 | .83 ± .07 | 1.13 ± .13 | 1.33 ± .08** | 1.30 ± .16** | 1.20 ± .18 | 1.27 ± .14* | 1.35 ± .15** |
| 70 | 1.08 ± .07 | 1.32 ± .13 | 1.40 ± .18* | 1.32 ± .06 | 1.27 ± .09 | 1.26 ± .07 | 1.43 ± .19 |
| 100 | .88 ± .03 | 1.50 ± .11** | 1.17 ± .13* | 1.08 ± .08 | 1.08 ± .09 | 1.12 ± .11 | 1.13 ± .10 |
| 130 | 1.11 ± .10 | 1.58 ± .19** | 1.38 ± .14* | 1.23 ± .08 | 1.10 ± .07 | 1.13 ± .10 | 1.13 ± .08 |
| 160 | 1.17 ± .08 | 1.48 ± .12 | 1.58 ± .09* | 1.40 ± .07 | 1.25 ± .09 | 1.32 ± .12 | 1.30 ± .07 |
| 190 | 1.13 ± .10 | 1.27 ± .10 | 1.32 ± .14 | 1.28 ± .09 | 1.13 ± .10 | 1.20 ± .10 | 1.22 ± .07 |
| 220 | 1.02 ± .06 | 1.23 ± .15 | 1.17 ± .16 | 1.15 ± .05 | 1.03 ± .08 | 1.02 ± .09 | 1.32 ± .10 |
| 250 | 1.25 ± .14 | 1.50 ± .10 | 1.42 ± .10 | 1.43 ± .10 | 1.34 ± .14 | 1.35 ± .17 | 1.33 ± .12 |

a Data presented are least-squares means ± SE.

*p<.05 (Compared with d -2 value).

**p<.01 (Compared with d -2 value).

Figure Legends

- Figure 1. Average body and paired testes and epididymides weight of boars between 40 and 250 d of age.
- Figure 2. Profile of mean (+SE) serum E₂ concentrations of boars between 40 and 250 d of age.
- Figure 3. Profile of mean (+SE) serum C concentrations of boars between 40 and 250 d of age.
- Figure 4. Profile of mean (+SE) serum LH concentrations of boars between 40 and 250 d of age.

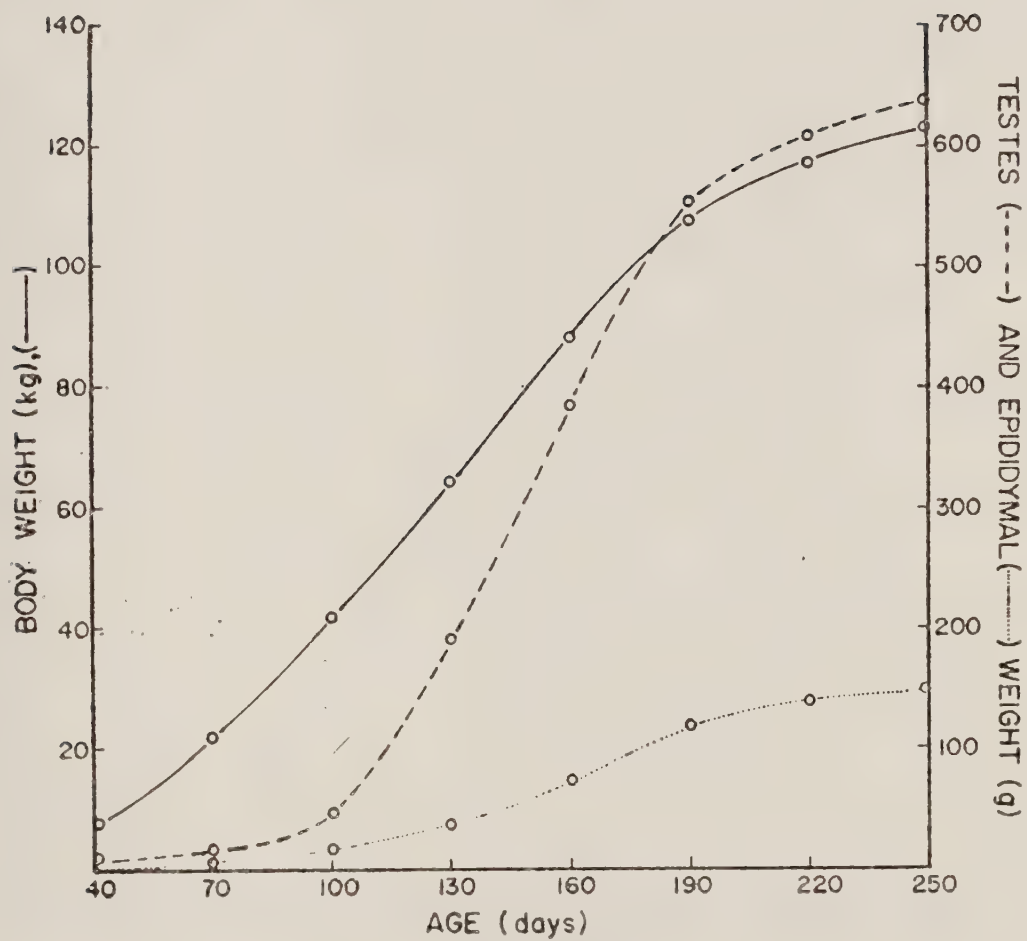


Figure 1. Average body and paired testes and epididymides weight of boars between 40 and 250 days of age.

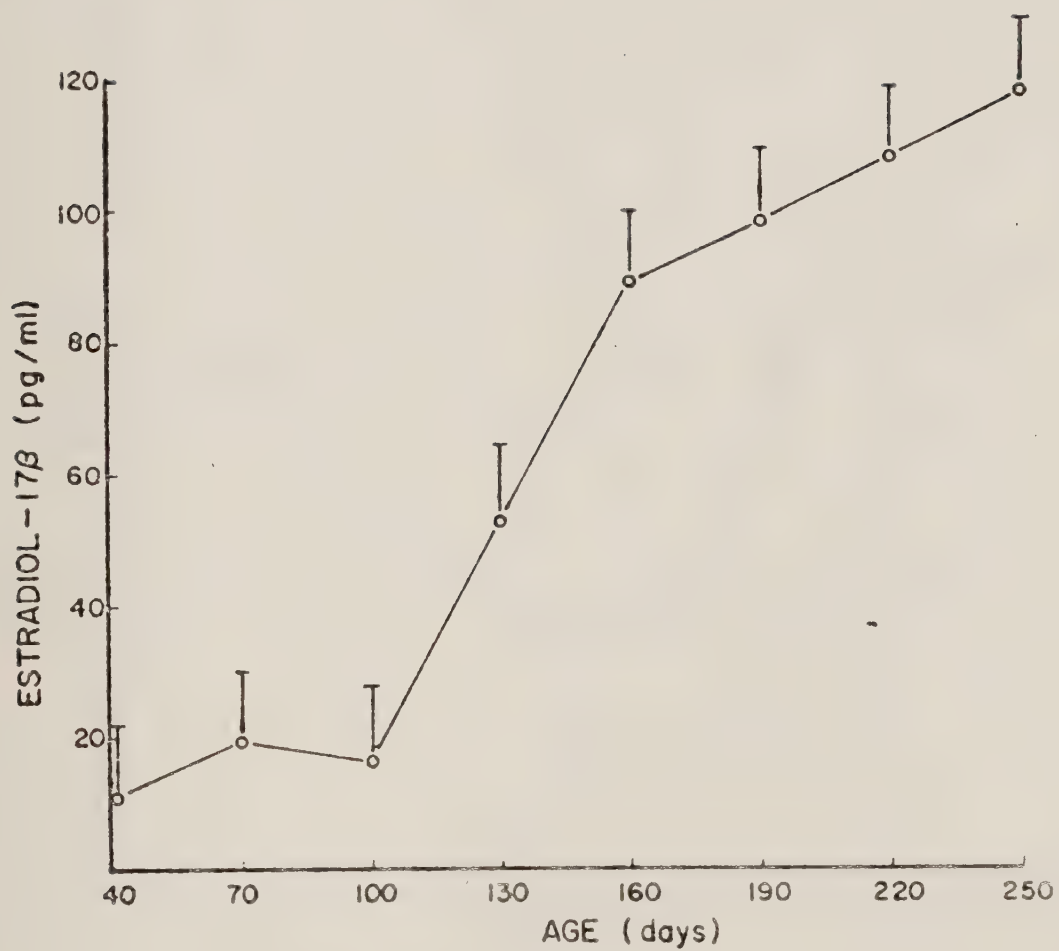


Figure 2. Profile of mean (+SE.) serum E_2 concentrations of boars between 40 and 250 days of age.

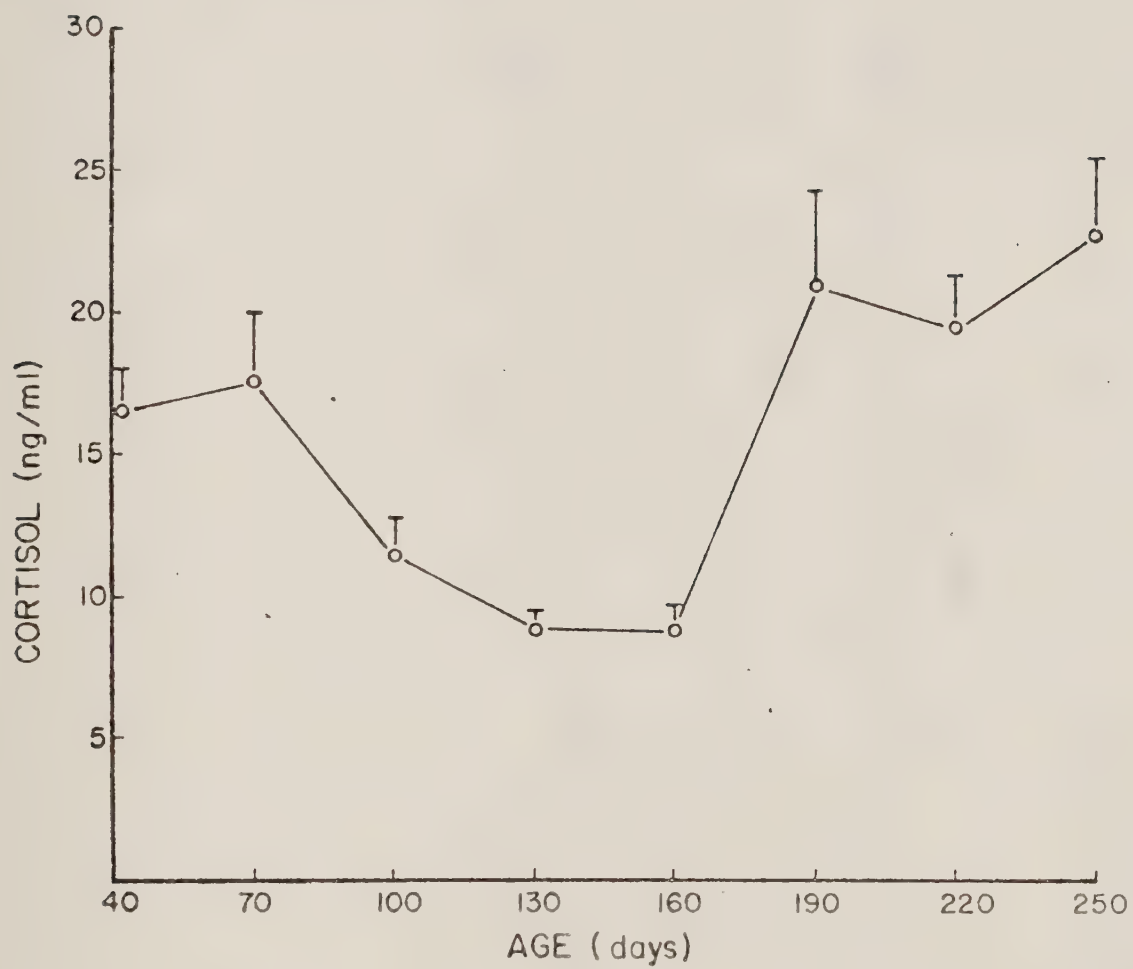


Figure 3. Profile of mean (+SE.) serum C concentrations of boars between 40 and 250 days of age.

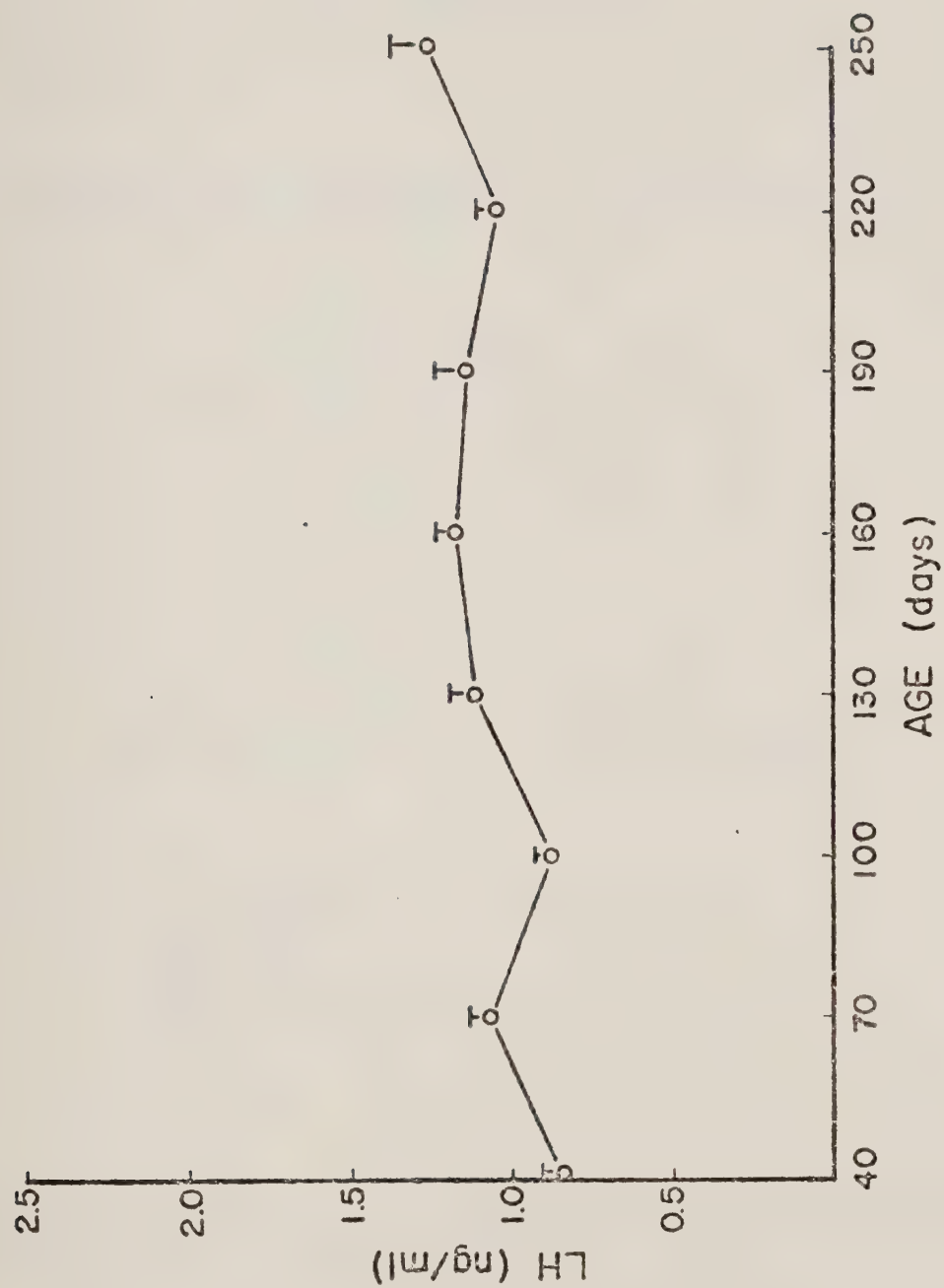


Figure 4. Profile of mean (+SE.) serum LH concentrations of boars between 40 and 250 days of age.

PUBERTAL DEVELOPMENT OF THE BOAR: AGE-RELATED CHANGES IN TESTICULAR
MORPHOLOGY AND IN VITRO PRODUCTION OF TESTOSTERONE
AND ESTRADIOL-17 β ^{1,2}

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Abstract

The responsiveness of testicular tissue, in terms of testosterone (T) and estradiol-17 β (E₂) production, to human chorionic gonadotropin (hCG) stimulation in vitro was assessed during pubertal development of the boar. A morphometric investigation was conducted concurrently to quantitate Leydig cell and seminiferous tubule changes in the testes of developing boars. Since whole tissue preparations were incubated, the morphometric investigation allowed for expression of T production on a Leydig cell basis while E₂ production was expressed per 500 mg of testicular tissue. Testicular volume percentage of seminiferous tubules increased from 36% at 40 days of age to a maximum of 72% at 190 days of age. Increases in tubular diameter were from 65 μ m at 40 days of age to 236 μ m at 250 days of age. Testicular volume percentage of Leydig cells decreased from 40% at 40 days of age to 10% at 250 days of age. Measurable increases in capacity and sensitivity of T and E₂ production were detected during pubertal development. The capacity of Leydig cells for T production and testicular tissue for E₂ production was greatest ($P < .05$) after hCG stimulation in boars that were 130 and 160 days of age. In addition, sensitivity, as judged by the regression coefficient of T and E₂ production on log dosage of hCG was greater ($P < .05$) for T and tended to be greater ($P < .10$) for E₂ in testicular tissue from boars 130 days of age. The data presented support the hypothesis that one factor in pubertal development of boars is an increased capacity and sensitivity of the testes to gonadotropin stimulation.

Introduction

Since only minor, if any, elevations occur in LH concentrations during pubertal development in the boar (Romanowicz et al., 1976; FlorCruz and Lapwood, 1978; Allrich et al., 1981), alterations in gonadal sensitivity to gonadotropins may be partially responsible for elevated concentrations of testosterone (T) (Colenbrander et al., 1978; FlorCruz and Lapwood, 1978; Lapwood and FlorCruz, 1978; Tan and Raeside, 1980; Allrich et al., 1981) and estradiol-17 β (E₂) (Allrich et al., 1981) observed during this period. Elevated T and E₂ are associated with growth and function of target tissue, most notably that of seminiferous tubules and accessory sex glands.

A current hypothesis (Odell et al., 1974; Odell and Swerdloff, 1975; Payne et al., 1977) states that there are changes in testicular responsiveness to LH as pubertal development progresses in the male rat. The changes in responsiveness are usually monitored by quantifying T production after gonadotropin stimulation in vivo or in vitro. No reported studies have comprehensively investigated developmental changes in T and E₂ production by incubated porcine testicular tissue after stimulation with a gonadotropin. The objectives of the present study were to characterize morphological changes in the testes and determine whether or not changes occur in maximal T and E₂ production and/or sensitivity of testicular tissue to a gonadotropin during pubertal development in the boar.

Materials and Methods

Forty-eight Landrace X Duroc boars were reared in a total confinement environment. Boars were exposed to 12 h of light daily until 10 weeks of age and 16 h of light daily thereafter. During rearing, boars were maintained in homosexual groups but were never isolated from possible audible and pheromo-

nal stimuli of contemporary females. At weaning, boars were randomly allotted in a randomized block design with blocking on sire groups (six sires used) to eight castration ages (40, 70, 100, 130, 160, 190, 220 and 250 days).

At castration, anesthesia was induced and maintained with sodium thiopental iv. Testes and epididymides were promptly removed from boars and weighed separately. Within 15 min after castration, the parenchymal tissue of the right testis was dissected free and randomly cut into 500 mg pieces which were minced in separate 25 ml Erlenmeyer flasks containing 5 ml of TC 199 buffered with Hepes (N-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid) at a final concentration of 25 mM. Following a 1/2 h preincubation period in a shaking waterbath (36°C) in an atmosphere of 95% O₂ and 5% CO₂, the media was carefully decanted and replaced with 5 ml of TC 199 (25 mM Hepes) containing 0, 5, 25, 125, 625 or 3125 mIU human chorionic gonadotropin (hCG)/ml media with dosages being triplicated. A time-course study was then conducted with subsamples (100 µl) of the media collected at 1/2, 1, 2 and 3 h after initiation of the incubation procedures with hCG. Media samples were frozen at -20°C until assayed for T and E₂. The 1/2 h preincubation was necessary, because large quantities of T and E₂ were released into the media at mincing and tended to mask T and E₂ production when stimulation with hCG commenced.

The entire left testis was immediately perfused, via the testicular vein, with cacodylate-buffered 1% glutaraldehyde-3% formaldehyde at room temperature (Johnson and Neaves, 1981). The perfused testis was then stored on ice and transported to the laboratory where 2 mm³ pieces were taken from the top, middle and bottom 1/3 of the testis and further fixed for at least 2 h in cacodylate-buffered 2% glutaraldehyde. The pieces were then postfixed overnight in osmium

tetroxide, dehydrated in ethanol and embedded in araldite. Sections of testis were cut at a thickness of 2 μ m and stained with toluidine blue and basic fuchsin (Sata and Shamoto, 1973; Alsop, 1974).

Approximately 100 cross-sections of seminiferous tubules and associated interstitial tissue were examined per boar. Volume percentages of selected structures were determined by means of the point raster method (Chalkley, 1943); using a 456 point raster on 8 x 10 inch photomicrographs. Total magnification was 463 X and 367 X for tissue sections from boars less than or equal to 100 days and greater than 100 days of age, respectively. In addition, seminiferous tubule diameters and number of Leydig cells per unit area were determined on the same photomicrographs.

Leydig cells were assumed to be spherical and therefore the area occupied by Leydig cells on a photomicrograph was assumed to be made up of circles. Area of Leydig cells per photomicrograph was divided by the number of Leydig cells in that area. Using the diameter determined from the average area of a Leydig cell, the volume of Leydig cells was calculated for each boar (Abercrombie, 1946). The number of Leydig cells per 500 mg piece of testicular tissue for each boar was estimated by the following equation:

$$\text{Number of Leydig cells} = \frac{(5 \times 10^{11} \mu\text{m}^3) (\text{volume percentage of Leydig cells})}{\text{Leydig cell volume}}$$

Tissue density was assumed to be unity. Testosterone production per Leydig cell was then calculated by dividing total T produced in the media by the number of Leydig cells determined in 500 mg of testicular tissue. The E₂ production was expressed as total E₂ produced in the media per 500 mg of testicular tissue.

Maximal T production was defined as the total T produced per Leydig cell after 1 h of stimulation with 3125 mIU hCG/ml media. Maximal E₂ production was

defined as the total E₂ produced per 500 mg testicular tissue after 3 h of stimulation with 3125 mIU hCG/ml media. Sensitivity of testicular tissue to hCG stimulation was determined as the regression coefficient of T and E₂ production on log dosage of hCG.

Concentrations of T were determined in triplicate 10 µl aliquots of media by radioimmunoassay (RIA) as described by Ford et al. (1980) utilizing an antiserum developed in rabbits (Cambridge Nuclear Radiopharmaceutical Corporation-248) which had less than 10 % crossreactivity with dihydro-testosterone. Samples were assayed without extraction. Concentrations of T were measured against standard curves of T (Sigma Co., St. Louis, MO) which ranged from 10 to 2000 pg. Intraassay and interassay coefficients of variation were 7.0 and 9.2%, respectively.

Concentrations of E₂ were determined in duplicate 10 µl aliquots of media by RIA as described by Kesler et al. (1977) utilizing an antiserum provided by Dr. Norman Mason (Eli Lilly Co.). Samples were assayed without extraction. Concentrations of E₂ were measured against standard curves of E₂ (Sigma Co., St. Louis, MO) which ranged from .78 to 200 pg. Intraassay and interassay coefficients of variation were 5.5 and 7.5%, respectively.

The data were analyzed by least-squares fixed-model procedures (General Linear Models procedure; Barr et al., 1979). The model involved a split-split plot analysis with age as main plot, hCG dosage as subplot and time of incubation as sub-subplot. Where heterogeneity of variance existed, log transformation of data preceded analysis. Differences between treatment means were determined by the Newman-Keuls test (Steel and Torrie, 1960).

Results

Mean body weights and paired weights of testes and epididymides increased throughout the period of sampling from 40 to 250 days of age (Table 1). Within age group, neither testes nor epididymides weight was significantly correlated with body weight ($r = .50$, $P > .30$).

Testicular volume percentage of seminiferous tubules and tubular diameters increased ($P < .01$) with age (Fig. 1). At 40 and 70 days of age, volume percentages of seminiferous tubules were similar and steady increases occurred thereafter to 190 days of age with values similar at 220 and 250 days of age. Seminiferous tubular diameter increased similarly. The largest increase occurred between 100 and 130 days of age and reached a maximum value of 236 μ m at 250 days. No regional differences were detected in testicular volume percentage or diameter of seminiferous tubules.

Volume percentage of Leydig cells (Fig. 2) in the testes had a reverse trend. Maximal values (45 %) were at 70 days of age. Volume percentage of Leydig cells then declined in a step-wise fashion until reaching 10 % at 220 days. Similar values were observed at 250 days of age. Volume per Leydig cell showed no systematic age-related alterations except for a marked elevation ($P < .05$) at 160 days of age. No regional differences were detected in testicular volume percentage or volume of Leydig cells.

Total weight of Leydig cells per paired testes increased to a maximum at 160 days of age and then decreased slightly thereafter (Table 1). In contrast, total number of Leydig cells per paired testes increased through 250 days of age. However, total number of Leydig cells appeared to stabilize briefly between 130 and 160 days of age before increasing again at 190 days of age.

Increasing dosages of hCG elicited increased ($P < .01$) production of T and E_2 for all ages (Tables 2 and 3, respectively). The time-course of T production was such that maximal responses were obtained by 1 h of incubation. In contrast, E_2 production increased through 3 h of incubation. No age differences were detected in T or E_2 time-course production.

Maximal production (defined as steroid production at 3125 mIU hCG/ml media), of both T and E_2 , was greatest ($P < .05$) at 130 and 160 days of age (Tables 2 and 3, respectively). Sensitivity of steroid production, as judged by the calculated ED_{50} parameter, tended to increase ($P < .10$) after 100 days of age for both T and E_2 production (Tables 2 and 3, respectively).

Discussion

Changes in body, testes and epididymides weight were similar to those reported previously (Phillips and Andrews, 1936; McKenzie et al., 1938; Swierstra, 1976; van Straaten and Wensing, 1977). Within subclass correlations yielded no significant correlations between body weight and paired testes or epididymides weight indicating that body weight is not a primary factor controlling testes growth.

Increases seen in testicular volume percentage of seminiferous tubules and seminiferous tubular diameters with age agree with those reported elsewhere (Palmer et al., 1971; van Straaten and Wensing, 1977). The large increases in volume percentage and diameter of seminiferous tubules that occurred between 100 and 160 days reflects, in part, the profound alterations occurring in testicular morphology during this period which culminates in the release of spermatozoa into the seminiferous tubule lumen. The expansive growth of the seminiferous tubules may be caused by the action of androgens being secreted by the Leydig cells at this time.

In accordance with the expanding volume occupied by seminiferous tubules, Leydig cells decreased in volume percentage as the age of the boar increased. Other researchers (van Straaten and Wensing, 1977, 1978) have noted such changes. The greatest decrease in volume percentage of Leydig cells occurred between 100 and 190 days of age which corresponds with the greatest increase in volume percentage of seminiferous tubules. Although volume percentage of Leydig cells decreased, the total number of Leydig cells within the testis increased with age due to the immense growth of the testis during pubertal development (van Straaten and Wensing, 1978). In contrast, total weight of Leydig cells did not increase steadily throughout development but rather reached a maximum at 160 days of age. The relationship of total Leydig cell weight and serum steroid concentrations need careful evaluation. The changes observed in Leydig cell volume are similar to those reported by van Straaten and Wensing (1978). The increase in Leydig cell volume at 160 days of age may partially be explained by the apparent reduction of mitotic division of Leydig cells and by Leydig cell growth from 130 to 160 days of age resulting in increased Leydig cell volume.

The morphometric study conducted with the perfused testis was considered the appropriate method to monitor compositional changes in the testicular tissue that was incubated. It has been shown (Kennelly and Foote, 1962) that both testes of the boar normally develop in synchrony.

The present study demonstrates an enhanced steroidogenic response, as measured by T and E₂ production, by testicular tissue in vitro to hCG stimulation during pubertal development. E₂ production was expressed per 500 mg of testicular tissue because E₂ synthesis in the testes probably involves the interaction of Leydig cells and seminiferous tubules (Dorrington et al.,

1976; Steinberger et al., 1979). Testosterone production per Leydig cell and E₂ production per 500 mg of testicular tissue increased at all hCG dosages at 130 and 160 days of age, indicating that this period in the life of the boar may be important in determining potential steroid production. The sensitivity of steroid production appeared to increase after 100 days of age, again indicating that this period may be critical for steroid production by the testes.

Previous studies (Wrobel et al., 1973, 1974; Tonhardt et al., 1976) with porcine testicular tissue have indicated that steroidogenesis increases at or near puberty in the boar. Recent evidence (Peyrat et al., 1981; Berardinelli et al., 1982) indicates that changes in LH receptor numbers of Leydig cells do not play a primary role in this response.

Examining pubertal development of bulls, McCarthy et al. (1979) failed to detect major changes in T production from in vitro stimulation of testicular tissue with LH. However, their data were expressed relative to incubated testicular protein and differences due to testicular compositional changes, that are known to occur during puberty (Phillips and Andrews, 1936), were not accounted for. Studies with rats (Ficher and Steinberger, 1971; Odell et al., 1974; Wiebe, 1976; Purvis et al., 1978) and rabbits (Chubb et al., 1978) have indicated increased testicular steroidogenesis during pubertal development.

The evidence presented supports the hypothesis that testicular sensitivity to gonadotropin stimulation increases during pubertal development. The increases in maximal T and E₂ production and gonadotropin sensitivity is reflected, in part, by elevations in serum T and E₂ concentrations at this time (Colenbrander et al., 1978; FlorCruz and Lapwood, 1978; Lapwood and

FlorCruz, 1978; Claus and Hoffman, 1980; Tan and Raeside, 1980; Allrich et al., 1981).

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Table 1. Body weights, paired testes and epididymides weights, and total weight and total number of Leydig cells per paired testes of boars between 40 and 250 days of age.

| Age (days) | No. of boars | Body weight (kg) | No. of boars | Testes weight (g) | Epididymides weight (g) | Total weight of Leydig cells (g) | Total number of Leydig cells ($\times 10^{-6}$) |
|------------|--------------|----------------------------|--------------|-------------------|-------------------------|----------------------------------|---|
| 40 | 48 | 8.7 \pm 0.3 ^a | 6 | 8.2 \pm 1.1 | 2.8 \pm 0.2 | 3.30 \pm .4 | 1,714 |
| 70 | 42 | 22.5 \pm 0.6 | 6 | 21.9 \pm 2.4 | 8.5 \pm 1.1 | 9.86 \pm 1.1 | 5,247 |
| 100 | 36 | 41.4 \pm 0.7 | 6 | 49.1 \pm 5.1 | 20.3 \pm 1.2 | 18.76 \pm 1.9 | 13,717 |
| 130 | 30 | 66.0 \pm 1.2 | 6 | 202.9 \pm 23.5 | 42.0 \pm 2.9 | 47.48 \pm 5.5 | 23,252 |
| 160 | 24 | 90.0 \pm 1.9 | 6 | 407.7 \pm 49.2 | 78.8 \pm 5.6 | 79.90 \pm 9.6 | 23,809 |
| 190 | 18 | 108.8 \pm 1.6 | 6 | 564.5 \pm 36.3 | 121.9 \pm 4.6 | 77.90 \pm 5.0 | 44,031 |
| 220 | 12 | 116.9 \pm 1.8 | 6 | 612.8 \pm 34.9 | 143.2 \pm 8.0 | 61.28 \pm 3.4 | 55,029 |
| 250 | 6 | 123.0 \pm 2.2 | 6 | 638.5 \pm 43.9 | 147.2 \pm 5.6 | 67.68 \pm 4.7 | 56,060 |

^a Data presented are least-squares means \pm SE.

Table 2. Effect of hCG on testosterone production (fg/Leydig cell), after 1 h incubation, by testicular tissue from boars between 40 and 250 days of age.

| Age (days) | No. of boars | hCG dosage (mIU/ml media) | | | | | |
|---------------|-----------------|---------------------------|-------|-------|-------|-------|-------|
| | | 0 | 5 | 25 | 125 | 625 | 3125 |
| 40 | 6 | 2.1 | 5.7 | 8.4 | 12.6 | --- | 17.5 |
| 70 | 6 | 2.9 | 5.1 | 7.4 | 9.5 | 13.5 | 17.0 |
| 100 | 6 | 2.9 | 4.4 | 5.9 | 8.2 | 11.8 | 15.2 |
| 130 | 6 | 8.5 | 21.0* | 29.0* | 34.8* | 42.5* | 45.7* |
| 160 | 6 | 10.7 | 16.3* | 22.4* | 28.0* | 34.6* | 42.5* |
| 190 | 6 | 7.0 | 10.5 | 11.8 | 16.0 | 17.9 | 19.8 |
| 220 | 6 | 8.5 | 12.8 | 16.6 | 20.1 | 26.2 | 26.6 |
| 250 | 6 | 8.5 | 11.3 | 12.8 | 15.3 | 18.6 | 20.1 |

a Data presented are least-squares means.

* These values are greater ($P < .05$) than other values within the same column.

Table 3. Effect of hCG on estradiol-17 β production (ng/500 mg testicular tissue), after 3 h incubation, by testicular tissue from boars between 40 and 250 days of age.

| Age (days) | No. of boars | hCG dosage (mIU/ml media) | | | | | |
|---------------|-----------------|---------------------------|-------|-------|-------|-------|--------|
| | | 0 | 5 | 25 | 125 | 625 | 3125 |
| 40 | 6 | 7.2 ^a | 7.7 | 8.9 | 12.2 | --- | 22.7 |
| 70 | 6 | 14.0 | 17.1 | 23.3 | 31.0 | 40.5 | 47.9 |
| 100 | 6 | 12.0 | 16.6 | 17.8 | 21.4 | 30.8 | 45.9 |
| 130 | 6 | 22.1 | 35.0* | 46.9* | 71.8* | 89.6* | 102.5* |
| 160 | 6 | 17.6 | 25.1* | 31.9* | 40.8* | 51.9* | 62.3* |
| 190 | 6 | 13.9 | 17.2 | 20.8 | 25.8 | 30.7 | 37.7 |
| 220 | 6 | 12.6 | 17.7 | 19.3 | 23.8 | 26.2 | 27.6 |
| 250 | 6 | 14.5 | 16.2 | 18.7 | 23.0 | 27.3 | 29.4 |

^a Data presented are least-squares means.

* These values are greater ($P < .05$) than other values within the same column.

Table 4. In vitro sensitivity of testicular tissue to hCG stimulation.

| Age (days) | No. of boars | Regression coefficient of hormone production on log dosage of hCG | |
|---------------|-----------------|--|------------------------------|
| | | Estradiol-17 β at 1h | Testosterone at .5h |
| 70 | 6 | 5.0 \pm 1.5 ^b | 2.7 \pm .5 ^b |
| 100 | 6 | 4.4 \pm 2.6 ^b | 3.1 \pm .9 ^b |
| 130 | 6 | 11.2 \pm 4.7 ^a | 10.2 \pm 1.7 ^a |
| 160 | 6 | 7.3 \pm 1.5 ^{a,b} | 7.1 \pm 2.1 ^{a,b} |
| 190 | 6 | 4.8 \pm 1.7 ^b | 2.4 \pm .5 ^b |
| 220 | 6 | 3.2 \pm .2 ^b | 3.7 \pm .8 ^b |
| 250 | 6 | 2.8 \pm .4 ^b | 3.4 \pm .9 ^b |

a,b Means \pm SE with different superscripts differ for estradiol-17 β (ng E₂/500 mg of testicular tissue), P<.10 and for testosterone (fg T/leydig cell), P<.05.

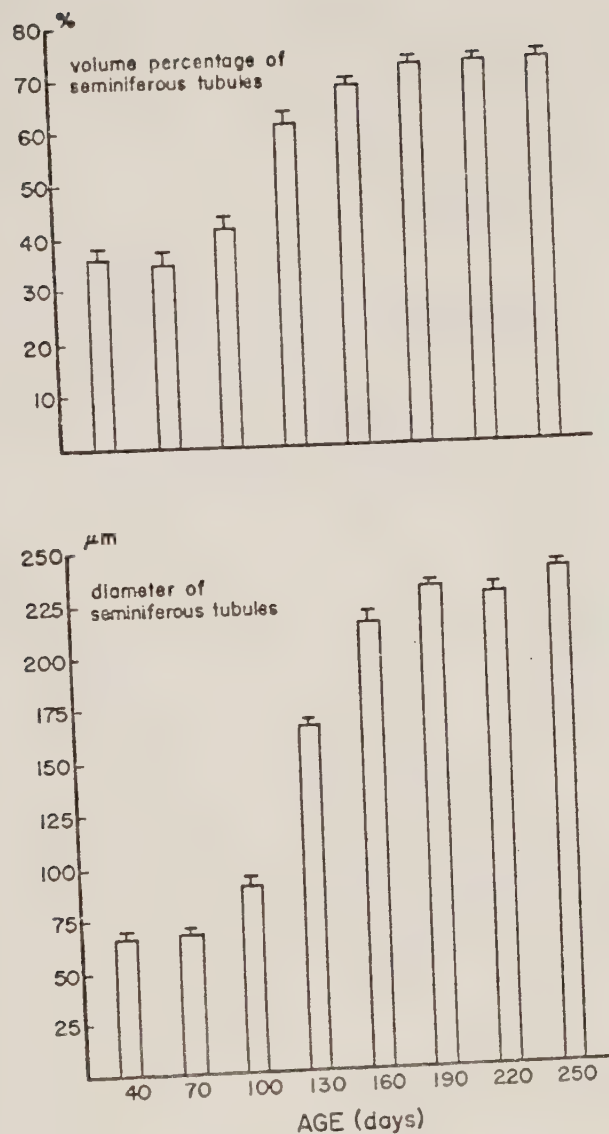


Fig. 1. Testicular volume percentage (+SE.) and diameter (+SE.) of seminiferous tubules of boars between 40 and 250 days of age.

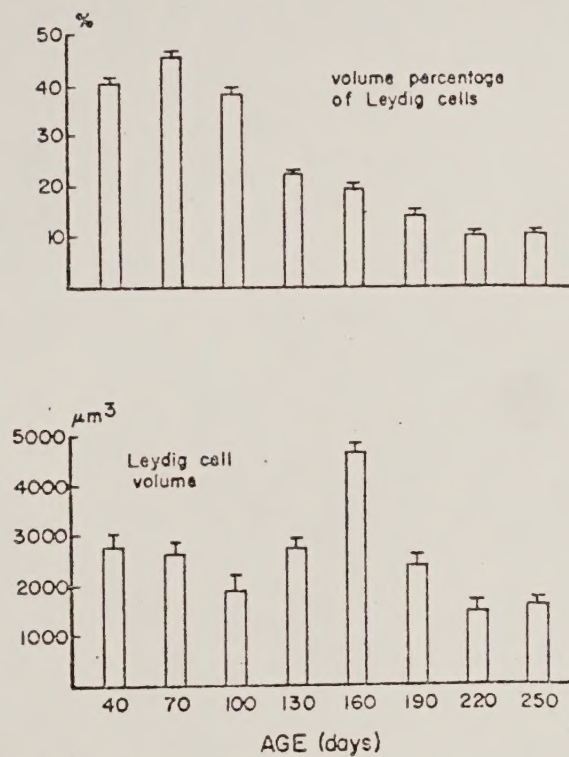
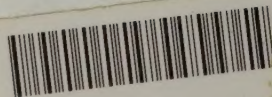


Fig. 2. Testicular volume percentage (+SE.) and volume (+SE.) of Leydig cells of boars between 40 and 250 days of age.



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